DETERMINING THE DIGESTIBLE LYSINE REQUIREMENT OF 22 TO 47 WEEK-OLD LOHMANN LAYING HENS USING TWO REQUIREMENT TITRATION METHODOLOGIES

BY

HALEY LYNN SPANGLER

THESIS

Submitted in partial fulfillment of the requirements
for the degree of Master of Science in Animal Sciences
in the Graduate College of the
University of Illinois at Urbana-Champaign, 2015

Urbana, Illinois

Adviser:

Professor Carl M. Parsons

ABSTRACT

Over the past 15 to 40 years, genetic improvements have been made to obtain higher egg production for commercial laying hens. In addition to genetic progress, there have been substantial changes in environmental management, cage density and feed ingredient quality. These changes have resulted in more efficient bird, and there is evidence that their nutrient requirements may have changed. Lysine (Lys), is an essential and usually the second limiting amino acid (AA) in poultry diets and its requirement is especially important because it is used as the reference AA when utilizing the ideal protein concept in feed formulation. When using this method, it is very important that the Lys requirement be accurate; failure to do so may result in errors in requirements for all other AAs included in the diet. Therefore, a study was conducted in order to determine the digestible Lys (DLYS) requirement of modern day laying hens using two different titration methods. One thousand six hundred and eighty Lohmann LS Lite caged hens were allotted to 15 dietary treatments using increasing crude protein (CP) or constant CP DLYS titration methodologies from 22 to 47 weeks of age. From Week 0 to 11 for the increasing CP series, the dietary DLYS and CP levels increased from 0.565 to 0.980% and 13.8 to 21.7%, respectively. At Week 12, DLYS levels were decreased to .468to.845% and CP levels were decreased to 12.2 to 19.3%; these levels were fed for the remainder of the experiment. For the constant CP series, DLYS levels increased from 0.565 to 0.980% while CP remained relatively constant at 16%. Again, at Week 12 of the trial, DLYS levels were decreased to range from 0.468 to 0.845%, with a constant CP at 14%. An industry control diet with a DLYS level of 0.807% and 18.5% CP was fed for Weeks 0 to 11. At Week 12, the DLYS and CP levels were adjusted to 0.688% and 15.3%, respectively. Increasing DLYS had a significant effect ($P< 0.05$)

on egg production, egg weight, egg mass and feed efficiency for both titration methods. However, increasing DLYS generally had no significant effect on percentage of egg yolk, white and solids. Broken line regression, the maximum of the quadratic polynomial (QP max) regression, and the intercept of the broken line and QP were calculated and used to estimate the DLYS requirement for egg production, egg mass, and feed efficiency. Broken line regression consistently yielded the lowest requirements and QP max regression yielded the highest, with the intersection of the broken line and QP max method yielding an intermediate requirement estimate. For example, when using the increasing CP titration method, the DLYS requirements for egg mass were 655, 817 and 706 mg/hen/d for the broken line, QP max, and the intercept of the broken line and QP regression methods, respectively. The DLYS for egg production was generally lower than that for egg mass and feed efficiency. When using the constant CP titration method, the DLYS requirements for egg mass were 703, 863 and 772 mg/hen/d for the broken line, QP max, and the intercept of the broken line and QP regression methods, respectively. The DLYS requirement estimated using the constant CP titration method were more variable and less precise than the requirements estimated using the increasing CP titration method.

Keywords: digestible lysine, laying hens, broken line regression, quadratic polynomial regression

For my parents and fiancé,

Sharon and Mike Spangler and Jeremiah,

for the endless love and support

ACKNOWLEDGEMENTS

First, I would like to express my appreciation to my adviser Dr. Carl Parsons, the support, kindness, friendship and knowledge that you have bestowed upon me is unmatched by any other individual in my life. I cannot thank you enough for all the conversations that we have had, whether it's regarding my thesis work or facing personal decisions. Your wisdom has made me a better person.

I would also like to thank my committee member, Dr. Ken Koelkebeck, for taking time to talk to me when I was an undergraduate and sparking my desire to work with poultry. I owe you so much more than you know. I offer my sincere gratitude and thanks to my committee member, Dr. Paul Tillman, for all the wisdom and expertise that you have given me throughout this process.

This research would not be possible without the help and support from my lab group and the Poultry Farm Staff. I would like to thank Pam Utterback and Sam Rochell for their help with my research project. I would like to express my appreciation to Christina Hanna, my fellow graduate student and friend. Your words of encouragement and kindness have been essential during graduate school and I can think of no other individual that I would rather feed hens, collect excreta, or study with. To my lab members, thank you all for your willingness to help feed such a large and long laying hen trial. You all have become great friends of mine, and I will miss our time in graduate school together.

I would like to thank my friends and family for their unwavering support and faith in me. My parents have given me every opportunity to succeed in life, and I consider myself blessed to be their daughter. The sacrifices you have made and the support you have shown have made me who I am today and I cannot thank you enough for each act of unconditional love. Many thanks to my siblings: Jason, Micah, and Kasey, for your unwavering love and support. Your confidence in me has allowed me to complete this part of my life journey. Thanks to my dearest friends who have always answered the phone when I needed to hear your encouragement the most, you have made a difference in my life that cannot be measured. To my future in-laws, thank you so much for the love you have extended to me throughout this process, I'm extremely lucky to have you all in my life.

Finally, I would like to give my love and thanks to my fiancé, Jeremiah, you have given me so much more than I could ever ask for. I am so overwhelmed with the endless supply of love, encouragement and happiness that you have given me. To be your wife will be my greatest accomplishment and I cannot wait to embark on many wonderful adventures with you.

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CHAPTER 1: INTRODUCTION

Although all 22 amino acids (AA) found in body proteins are physiologically essential, lysine (Lys) has no intermediary precursors; demanding that the requirement for Lys is met solely by dietary feed ingredients. Therefore, Lys is an essential AA used for bodily tissue, functions and protein accretion (Baker, 1997). Also, Lys is commonly the second limiting AA in poultry diets. Lys is generally a well-researched AA; however, published data conducted to determine the Lys requirement of laying hens may be outdated due to substantial genetic improvement over the last 15 to 40 years (Novak et al., 2004; Bregendahl et al., 2008). For example, the three studies used as a basis for the NRC (1994) Lys requirement of laying hens were conducted 26 to 34 years ago (Nathanael and Sell, 1980; Latshaw, 1981; Proudfoot, 1988).

In addition to being an essential AA and the second limiting AA in poultry diets, Lys is used as the reference AA in the ideal protein concept. The ideal protein concept was first described by Mitchell (1964) as a way to formulate poultry diets that met requirements using purified ingredients. In the current ideal protein formulation method, the Lys requirement is given a value of 100 and the requirement of the other AA are expressed as ratios to Lys. Thus, any errors in the Lys requirement will likely result in an error for the requirement of all other AA. Conducting research that estimates the requirement for AAs is difficult, mostly because analysis for AAs can be complicated and various factors can influence the estimation of AA requirements. These factors include, but are not limited to, environment, growth stage, genetics, and dietary factors. Lys, however, is easily analyzed and its importance to poultry production and diet composition allows for this AA to be used as the reference AA in the ideal protein concept (Baker et al., 2002). Therefore, knowing the digestible Lys requirement is vital when formulating laying hen diets for maximum production.

As discussed above, most of the studies conducted to determine the Lys requirement were conducted many years ago and most diets are now formulated based on ideal protein, making the accuracy of the Lys requirement very important. Therefore, the objective of this study was to determine the digestible Lys (DLYS) requirement of Lohmann LS Lite laying hens using two titration methodologies during 22 to 47 weeks of age. The first titration method allowed for increasing dietary crude protein (CP) as a result of the increase in DLYS, while the second method kept CP relatively constant and DLYS was increased by supplementing the diets with only L-Lys-HCL.

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CHAPTER 2: LYSINE REQUIREMENTS OF LAYING HENS AND REGRESSION METHODS: A LITERATURE REVIEW

Lysine requirements of laying hens for corn based diets

Usually Lys is the second limiting AA in corn-soybean meal diets and is the reference AA for the ideal protein concept. Therefore, determining the Lys requirement accurately is essential to formulating laying hen diets. A low protein diet with Lys supplementation is often used in order to establish a requirement estimate. One such study conducted by Harms and Waldroup (1963) had four dietary rations starting at considerably low protein levels (11, 13, 17 and 21%); Lys was supplemented at either 0.10% or 0.20% levels. Results from this trial indicated that at protein levels below 13%, Lys may be the first limiting AA instead of methionine (Met). At a more moderate protein level (17%) with AA supplementation, optimal production was achieved; a Lys requirement for this type of diet was suggested to be 494 mg/hen/d (Harms and Waldroup, 1963). Moran et al (1967) formulated a semi-purified diet to test the dietary deficiency of non-essential nitrogen and the minimal AA requirement for laying hens. This test diet contained 9.24% protein and was supplemented with 0.03% L-Lysine HCL. Any non-essential nitrogen deficiency was alleviated with supplementation of either 2.01%, 4.02%, or 6.03% diamonium citrate. Egg production, egg formation and maintenance needs were calculated in order to estimate both the essential and nonessential nitrogen requirements for laying hens. Significantly lowered egg production and body weight in all groups was observed, a likely reason was the nutritional inadequacy of the diet. Based on a 75% egg production, a body weight of 1.6 kg, an average egg weight of 62 g/egg, and a feed intake of 110 g/day, the Lys requirement was determined to be 454 mg/hen/d (Moran et al., 1967), which agreed well with Harms and Waldroup (1963).

Due to limited data on the Lys requirement, Bray (1969) conducted an experiment to determine the AA requirement again using low protein diets. A test diet containing a 60:40 corn and soybean blend was formulated with an initial protein level of 11.97%, with L-Lys-HCL then supplemented to obtain dietary Lys levels from 0.314% to 0.674% in order to observe the correlation between egg production and Lys intake. The results revealed a significant correlation between increased Lys and increased egg weight, body weight and egg yield. Least squares analysis of the egg yield data suggested a Lys requirement of 522 mg/hen/d (Bray, 1969).

Gleaves and Dewan (1970) conducted an experiment to determine Lys, Met, and tryptophan (Trp) requirements. Diets were formulated with increasing levels of L-Lys-HCL supplementation and a basal diet of 16 % protein. Feed consumption and production characteristics such as egg production and body weight gain of pullets fed the experimental diets were measured throughout the course of the experiment. Feed consumption was not affected by the Lys supplementation in either experiment, but egg production was numerically increased with the supplementation of Lys (as well as Met and Trp). Body weight gain also had a significant increase with Lys and Met supplementation. Based on the results of feed intake and egg production values with increasing Lys, Met, and Trp supplementation, a Lys requirement of 585 mg/hen/d was suggested by Gleaves and Dewan (1970).

Since the result of the earlier studies varied so greatly, likely due to differences in environment or strains of birds in each trial conducted, Pilbrow and Morris (1974) conducted an experiment to determine the Lys requirement in eight different stocks of laying hens. Mean body weight of the 63 pullets used for each stock and diet combination were 1.8 to 2.6 kg and peak egg output ranged from 43 to 51 g/bird/d. Statistical analysis of the production responses determined a significant, but small, difference among the stocks of laying hens in the amount of Lys required to produce one gram of egg. Using the relationship between egg production, Lys intake and body weight, an equation was developed to determine the optimal Lys requirement depending on stock. These Lys requirements ranged from 820 to 920 mg/hen/d, which is much higher than previously published data suggested (Pilbrow and Morris, 1974). With such large differences in published data, Sell and Johnson (1974) used corn-soybean meal and wheat-soybean meal diets in order to estimate a Lys requirement for laying hens using commonly available feedstuffs. Wheat and soybean meal rations contained 14% protein and were supplemented with large increasing levels of Lys in order to achieve a high and efficient rate of egg production. Corn and soybean meal rations contained 12 to 14% protein and again were supplemented with large increasing levels of AAs to obtain efficient production measurements. While hens fed wheat based diets did produce smaller eggs, the data suggested that the Lys requirement of hens fed wheat and corn based diets were similar and that an adequate Lys requirement would be 660 mg/hen/d (Sell and Johnson, 1974). A study by Latshaw (1976) agreed well with the Sell and Johnson (1974) study; they fed diets supplemented with Lys so that the Lys intake was 607 mg, 636 mg, or 657 mg. Based on this study and the results of other studies (Carlson and Guenthner, 1969; Jensen et al., 1974; Roberson, 1970) a Lys requirement of 650 mg/hen/d was determined to provide optimal egg production (Latshaw, 1976).

Hurwitz and Bornstein (1977) conducted three experiments using two models in order to test the Lys requirement for laying hens under various circumstances. The two models calculated the requirements based on AA supplementation and protein content of the diet in correlation with body weight, body weight gain, egg production rate and egg weight because according to the researchers, "protein and AA requirement are functions" of these production measurements (Hurwitz and Bornstein, 1977). Dietary protein ranged from 12.28 to 14.58%, and Lys supplementation ranged from 0.58 to 0.80%; diets were formulated with yellow corn and soybean meal. Rate of egg production ranged from approximately 75 to 81%, with an egg mass average of 48.65g throughout the course of the experiment. Based on the calculated requirements of their earlier work (Hurtwitz and Bornstein, 1973) and the data from this trial (Lys intake and production measurements), the Lys requirement was determined to be approximately 700 mg/hen/d (Hurtwitz and Bornstein, 1977). Overall, Lys requirements seem to be increasing over time, for instance, the NRC (1971) suggested a requirement of 500 mg/hen/d, whereas the NRC (1984) which used the results from Bray (1969) and several other studies, suggested a Lys requirement of 650 mg/hen/d.

More recent research to examine Lys requirement in laying hens includes the work of Schutte (1998) where corn based diets were used to estimate the Lys requirement of 24 to 36 week old White Leghorn strain hens. An egg mass yield of 57 g/hen/d was estimated to require approximately 900 mg of total Lys, this corresponded with a 720 mg per hen-d apparent fecal digestible lysine (Schutte and Smink, 1998). Production responses are vital when determining the Lys requirement, as well as other AA requirements, in laying hen diets. One such production response is egg component yield. Prochaska et al. (1996) determined Lys requirement based responses to egg weight, albumen weight, solids, egg production, yolk weight and more. At approximately 1,602 to 1,613 mg of Lys intake, optimal production responses for all parameters were met (Prochaska et al., 1996).

The current NRC (1994) published AA requirements for laying hens, a Lys requirement of 690 mg/hen/d was suggested based off of research that occurred decades ago (Nathanael and Sell, 1980; Latshaw, 1981; Proudfoot, 1988). More recently, Bregendahl (2008) conducted research to determine the AA requirements in 28 to 34 week old White Leghorn-type laying hens. Lys requirement, amongst other AAs requirements, were analyzed based on the response of body weight, feed utilization, egg mass, egg weight, and egg production. Broken line regression yielded results that the true digestible Lys requirement would be 538 mg/hen/d based on egg mass responses and 693 mg/ hen-d based on feed utilization (Bregendahl et al., 2008). In this paper, Bregendahl (2008) mentions that in the same strain of laying hens Coon and Zhang (1999) found that in 33 to 39 wk-old hens a Lys requirement of 706 mg/hen/d was determined and in 35 to 47 wk-old hens 636 mg/hen/d based on egg mass. Overall, more recent research indicates that the Lys requirement has changed greatly over the past 50 years.

Based on the above review it is apparent that the reported Lys requirement of laying hen varies greatly among studies. In earlier years, the reasons for such different requirements may be explained by the different strains or breeds of laying hens (Bustany and Elwinger, 1987). One such study was previously mentioned and was conducted using eight different stocks of laying hens to determine the Lys requirement; ultimately the Lys requirement was shown to range from 820 to 920 mg/hen/d corn based diets (Pilbrow and Morris, 1974). Other likely reasons for differences in Lys requirement among stocks are age of the hens used, specific production parameters measured, and genetic improvement in the laying hens over time (i.e. higher egg production and increased egg mass produced per day).

Lysine requirements of laying hens for wheat based diets

In earlier studies by Sell and Hodgson (1966), Thornton et al. (1957) and Waibel and Johnson (1961), it was reported that with the appropriate AA supplementation, 11 or 13% crude protein corn-soybean meal diets could yield optimum egg production. Also, Bray (1964) demonstrated good egg production could be obtained with low protein diets supplemented with Lys, Met, and Trp. However, little research to determine the Lys requirement when using wheat-soybean meal diets had been done. Therefore, two experiments were conducted with low and high protein diets supplemented with Lys and Met and using wheat as the major grain source (Sell and Hodgson, 1966). Hens fed 13.5% protein wheat-soybean meal diets had lower egg production than hens fed a 16.5% protein diet, and supplementation of diets with 0.1% Lys and 0.1% methionine hydroxy analogue (MHA) improved egg production by approximately 9%. Diets supplemented with DL-Met instead of MHA failed to exhibit a beneficial response to egg production and other performance parameters. Supplementing with only DL-Met or MHA depressed most production measurements, which suggests that Lys was the first limiting AA in these wheat-based diets. Thus, there may be a major difference in the AA balance in wheat vs. corn diets because Met is almost always the first limiting AA in corn-soybean meal diets. These researchers concluded that a Lys requirement of 620 mg/hen/d would be adequate for laying hens fed on wheat-soy diet (Sell and Hodgson, 1966).

March and Biely (1972) conducted a study with four experiments where laying hens were fed wheat based diets with herring meal or AA supplementation suggested a much higher Lys requirement. The results showed that in order to yield maximum egg mass, laying hens would need a daily Lys intake at approximately 800 to 850 mg/hen/d; however, researchers did not feel this was a practical Lys requirement and instead suggested a lower Lys requirement of 700 to

750 mg/hen/d (March and Biely, 1972). Bustany and Elwinger (1987) generally agreed with March and Bielys (1972) findings when they conducted a trial with two experiments using various strains of birds. The optimal egg output response curves suggested a Lys requirement ranging from 820 to 1023 mg/hen/d. A more economical and reasonable intake for Lys based on the strain of laying hen was calculated at approximately 766 to 956 mg/hen/d using the output response curve (Bustany and Elwinger, 1987).

This discussion clearly shows that the Lys requirement varies greatly among studies and this variation has left numerous researchers at a loss for recommending a specific Lys requirement. Comparing the use of corn and wheat diets in the same study, Jensen et al. (1974) suggested a Lys requirement ranging from 660 to 788 mg/hen/d. A higher Lys requirement was observed when hens were fed wheat rations; two possible reasons were proposed to explain the difference in Lys requirement based on corn vs. wheat-based diets. One reason may be that Lys is less available or digestible in wheat compared to corn; thus, more total Lys is needed to meet the requirement. The second reason is that the AA balance in wheat-based diets result in higher Lys requirement, again creating the need for more Lys supplementation (Jensen et al., 1974). Bray (1960) also stated that corn and soybean meal are usually the primary sources of AA, and a lack of these ingredients in diets may cause an AA deficiency. This agrees well with Sell and Hodgsons' work which suggested that in a wheat-soybean meal diet, Lys was the most limiting AA compared to a corn-soybean meal diet where Lys is commonly the second limiting AA (Sell and Hodgson, 1966). In more recent years, little research to determine the Lys requirement using wheat-based rations has been conducted. This is probably because laying hen AA requirement studies are mostly conducted in the U.S. where most diets are based on corn, not wheat.

Methods for estimating nutrient requirements

Among the various ways that nutritional requirements can be measured, the broken line model is one of the oldest methods utilized. Typically, the broken line method is characterized by being straight forward and yields an objective estimate for a nutrient requirement. The simplicity of this model makes it easy to estimate the requirement, since it consists of two segmented lines, one that has an increasing slope and the other that has zero slope. The intersection between the vertical and horizontal lines is considered as the breakpoint or the requirement (Robbins, 1986). Although equations may vary in some ways, the broken line regression model can be used to interpret a requirement using various AA intakes and production responses.

Since broken line regressions are based on the assumption that there is a linear correlation between AA intake and production response, the equation used for this regression is based on the production response as the function of the AA intake (Fisher et al., 1973). Robbins (1986) stated that in general there were two equations used for the broken line model, for a singular slope broke line the equation: $Y= L+ U(R-X_{LR})$ may be used and for a two slope broken line the equation $Y= L+U$ can be used. Noll and Waibel (1989) estimated a Lys requirement in growing turkeys at different environmental temperatures, using a broken line regression and also an exponential model. Although the exponential model yielded consistently higher Lys requirements than the broken line model, both models showed the same pattern of effect on environmental temperature on the Lys requirement (Noll and Waibel, 1989).

Broken line regression can be observed in an early paper by Bray (1965), where the requirement of Met was determined for young laying pullets. The requirement of Met was expressed as a percentage of the protein in the diet and in protein intake (g/d) of the laying hens. Bray (1965) found that in diets containing 7 and 10% protein, maximal egg yield was achieved with 2.44 and 2.35% Met, respectively. This corresponds to a protein intake of 11.33 g/hen/d, which Bray (1965) found to be lower than previously published data suggested. In a later paper, Bray (1969) again used the broken line regression to assess the requirement of several AAs. Egg yield (g/d) as the function of the Lys intake (mg/d) was calculated to have a Lys requirement that was higher than previously published data (Bray, 1969).

Russell and Harms (1999) used broken line regressions to estimate a requirement for tryptophan (Trp) in commercial laying hens. Egg production and content was regressed on the daily Trp intake of the laying hens to calculate a requirement that agreed well with previously published data (Russell and Harms, 1999). This same approach was used in another paper by Russell and Harms (2001), where the requirement of valine (Val) was estimated by regressing egg production, egg weight and egg content on Val intake (mg/hen/d). Faria et al., (2002) used broken line regressions to estimate the requirement of threonine (Thr) in commercial layers from 31 to 38 weeks and 45 to 52 weeks of age. Egg mass, calculated as egg production rate multiplied by egg weight, and egg production were regressed on Thr intake (mg/hen/d) to estimate a requirement for both age groups. For 31 to 38 week old hens, the broken line regression yielded a slightly higher requirement compared to hens aged 42 to 52 weeks, likely due to the decreased AA requirement as hens' age. Shivazad et al. (2002) reported an isoleucine (Ile) requirement for commercial layers starting at 35 weeks of age using broken line regression. Egg weight, production and mass were determined and were used to estimate the Ile requirement, which was lower than the NRC (1994) estimate.

More recently, Bregendahl et al., (2008) utilized the broken line method as described by Robbins (1986) in order to estimate a requirement for various AAs in Leghorntotype laying hens. Body weight change, feed utilization, egg mass, egg weight and egg production were used to determine the requirements of Met, Met+Cystine (Cys), Thr, Trp, Val, all relative to Lys according to the ideal AA profile. The ideal AA profile is a concept that uses Lys as the reference AA to express the requirement of other AA in laying hen diets (Baker et al., 2002). In general, most of the AA yielded requirements similar to the NRC (1994) (Bregendahl et al., 2008). It was noted that the AA requirements yielded from these studies may only be valid for the particular hens used and may not be appropriate for a commercial setting.

A general trend for AA requirement estimates based solely on the broken line regression to be slightly lower than previously published data might suggest is noticeable. However, there are several factors that may account for these discrepancies. In a paper by Faria et al. (2002) these authors reported that the variation in Thr requirement may be explained by Ishibashi et al., (1998) as an effect of several factors, including but not limited to, strain or age of hen, egg production rate and crude protein levels. Fisher and Morris (1970) reviewed various regression methods, in particular Brays' (1965) paper that used the broken line method to estimate the requirement. The estimate that Bray (1965) determined for the Met requirement was 223 mg/hen/d and was ultimately regarded as too low of a requirement by Fisher and Morris (1970). Fisher and Morris (1970) argued that since the maximum output of a response may be a continuous curve, the requirement could not be accurately determined from the broken line method, which fits two straight lines to a production output based on an AA intake.

Later, an extensive review on broken line regression was provided by Fisher et al. (1973) where two possible disadvantages of using the broken line regression method were discussed.

One disadvantage being that the model is based on the linear correlation between input of protein or AA intake and the output, or production of the laying hens. Secondly, the requirement estimate that is yielded from this method represents the average of the responses from individual hens (Fisher et al., 1973). If only the average requirement of the laying hens is being met, then a substantial amount of laying hens will either be overfed or underfed AAs.

While broken line regression is among the oldest and most straightforward methods used to estimate a nutritional requirements, it may not always be the most suitable to use. Another method that can be used to estimate AA requirements, as well as other nutritional requirements, is the quadratic polynomial. The quadratic regression method is mathematically more complicated than the broken line regression, and it also yields results that are more subjective. Objectively, the vertex of the quadratic curve is used to estimate the nutrient requirement; however, some lower value or percentage of the vertex is often selected which creates subjectivity.

Pilbrow and Morris (1974) used quadratic regression to compare the Lys requirement in eight stocks of hens since they felt that previously published data using broken line regression yielded inadequate nutrient requirements. The relationship between Lys intake, egg output, and body weight was expressed as a quadratic function. From the egg output (g/hen/d) response, Pilbrow and Morris (1974) determined a higher Lys requirement than the broken line requirement of Bray (1969). The latter difference may have been due to the different mathematical models used. However, it is also possible that the hens in the Bray (1969) study may have utilized Lys more efficiently, the available Lys in the basal diet might be higher than analyzed, or the analysis of the results was skewed since data included the initial period of the trial when hens may not have yet been AA deficient (Pilbrow and Morris, 1974).

Nathanael and Sell (1980) conducted an experiment to take quantitative measurements of the Lys requirement of laying hens. Data were collected from an experiment where increasing levels of dietary Lys were fitted to a polynomial equation in order to determine the Lys requirement. The response curve showed that maximum egg production was obtained at a daily Lys intake of 690 to700 mg/hen/d (Nathanael and Sell, 1980). More recently, Cardoso et al. (2014) conducted a study to determine the Thr requirement for white egg layers from 60 to76 weeks of age. Diets with increasing ratios of Thr:Lys were shown to have a quadratic effect on egg mass and production, which according to the authors, agreed well with previous research.

A likely reason that there was a difference in Lys requirement among studies using broken line versus quadratic regression models can be explained by the nature of the regressions used. Typically, quadratic regression estimates the requirement based on the vertex and yields a value that meets the requirement of all the hens in the population, whereas broken line regression determines the requirement of the average or mean of the population. Since the nature of the quadratic regression is to generally estimate a requirement that is high for most of the population, an estimate of 90 to 95% of the upper asymptote of the vertex of the quadratic curve is often used to determine a more reasonable requirement for practical feed formulations (Robbins, 1979). Schutte and Smink (1998) took a similar approach when estimating a Lys requirement, where they selected 90% of the maximum response as the suggested requirement. Whether 90% or 95% of the maximum response is used, it is an arbitrary number dependent on several factors that researchers must take into consideration. This may result in overestimation or underestimation of nutrient requirements depending on the population in question.

Fisher et al. (1973) discussed three methods to manipulate broken line regression so that a more reasonable requirement could be estimated. Among these methods, a model using both broken line and quadratic regression to estimate requirements for the average and individual population was discussed. By superimposing the two regressions onto each other and calculating the slope of the line that intersected the broken line and quadratic regression a more realistic requirement may be met. Fisher et al. (1973) calculated the slope of the line where the broken line regression and quadratic regression became tangent with one another. This line was defined as the limit of economic response at which point the cost of input and the gain of output were economical. It was suggested that a requirement under or over this estimation may yield an inadequate output (Fisher et al., 1973).

Baker et al. (2002) introduced a new method for estimating nutrient requirements where broken line and quadratic polynomial (QP) regression were fitted to the same data set for various AA requirements. In this study, weight gain or gain:feed was regressed onto either Trp, Thr, Ile, or Val by using broken line and QP. Baker et al. (2002) then calculated the first and second intercept for where the QP intersects with the broken line. The first intercept value yielded a requirement that was approximately 90% of the QP maximum (QP max) and was an objective way of determining nutrient requirements. Typically, an underestimation or overestimation of the requirement can be observed in broken line and QP max, respectively. However, when using the intersection of the broken line and the QP, Baker et al. observed a requirement that was an intermediate of the broken line and QP max estimates and represented a requirement that is more economical. Peganova et al. (2003) noted that the nutrient requirement of Trp may be more accurate by using the average of the broken line and QP, and thus used this similar approach when estimating the requirement.

Over the course of the last several years, genetic and production improvements have been made in laying hens. However, there has been very little research published to determine the digestible Lys (DLYS) of these modern laying hens. The accuracy of DLYS requirement is extremely important since it is used as the reference AA when formulating laying hen diets based on ideal protein or AA ratios. Therefore, this study was conducted to determine the DLYS requirement in Lohmann laying hens over a 25 week period using two different titration methodologies, either constant protein or increasing protein. The DLYS requirement was estimated using the broken line, the QP max, and the intersection of the broken line and QP so all these methods could be compared.

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CHAPTER 3: DETERMINING THE DIGESTIBLE LYSINE REQUIREMENT OF 22 TO 47 WEEK-OLD LOHMANN LAYING HENS USING AN INCREASING CRUDE PROTEIN TITRATION METHOD

ABSTRACT

A large laying hen trial was conducted from 22 to 47 weeks of age to determine the digestible Lysine (DLYS) requirement of laying hens by using an increasing crude protein (CP) titration method. Eight hundred and ninety six Lohmann LS Lite caged layers (22 weeks of age) were allotted to 8 dietary treatments and each treatment had 8 replications of 14 hens. The first 7 experimental diets initially contained DLYS levels increasing from 0.565 to 0.980% with respective protein levels increasing from 13.8 to 21.7%. Dietary Treatment 8 was a control diet which was calculated to contain 18.6% CP and 0.807% DLYS. These DLYS levels were reduced to 0.468 to 0.845% at Week 12 so that greater differences in production parameters could be obtained. Egg production, egg weight, egg mass and feed efficiency were calculated. Increasing DLYS levels had a significant ($P < 0.05$) effect on all of these production parameters. However, DLYS levels had a lesser effect on egg component measurements such as percentage of yolk, white and solids. Broken line regression, maximum of the quadratic polynomial (QP max) regression, and the intercept of the broken line and QP regressions were used to estimate the DLYS requirement. Broken line regression yielded the lowest requirement and QP max regression yielded the highest, with the intercept of the broken line and QP methods yielding an intermediate requirement estimate. For egg mass and feed efficiency, DLYS requirements 655 and 690, 817 and 866, and 706 and 778 mg/hen/d for the broken line, QP max, and the intercept of the broken line and QP models, respectively. The DLYS requirements were generally lower for egg production than for egg mass and feed efficiency. A DLYS requirement for egg

production was estimated to be 528, 727, and 620 mg/hen/d for the broken line, QP max, and the intercept of the broken line and QP, respectively.

Keywords: digestible lysine, crude protein, laying hens, broken line regression, quadratic polynomial regression

INTRODUCTION

In recent years, little research to determine the DLYS of laying hens has been done, even though there has been vast genetic improvement over the last 15 years (Bregendahl et al., 2008). Many (most) laying hen diets are formulated based on ideal protein or amino acid (AA) ratios. Lysine (Lys) is used as the reference AA when formulating diets based on the ideal protein profile (Baker, 1997). In addition, proper use of Lys and other AA can promote greater production efficiency and consequently a greater economic revenue since feed utilization can be improved (Silva et al., 2015). For these reasons, accurately determining the Lys requirement of laying hens during different production periods is essential in order to achieve maximum production.

The requirement for Lys, usually the second limiting AA in corn-soybean meal diets, has been determined by several different researchers with vast differences among requirement estimates. Early papers suggested a lower total Lys requirement of 494, 454 and 522 mg/hen/d (Harms and Waldroup, 1963; Moran et al., 1967; Bray, 1969). In more recent years, higher Lys requirements have been reported. For example, Schutte and Smink (1998) reported a DLYS requirement of 720 mg/hen/d and Prochaska et al. (1996) reported a total Lys requirement of 1,602 to 1,613 mg/hen/d when optimal production responses for all parameters were met. Bregendahl et al. (2008) determined ideal ratios of several AAs including Lys and estimated a Lys requirement of 538 mg/hen/d based on egg mass and 693 mg/hen/d based on feed utilization.

Based on the Reading model, Silva et al. (2015) reported a Lys requirement of 707, 660, and 669 mg/hen/d for egg mass to meet the requirement of approximately 97% of the population in the periods of 37 to 40, 41 to 44 and 45 to 48 weeks old hens, respectively. These estimates were similar to those obtained from a combination of the broken-line and QP models.

Lys requirements have been historically determined most often using broken line regression, a relatively objective method to estimating nutrient requirements. Several researchers have determined AA requirements of laying hens based on broken line regression for methionine (Bray, 1965), for Lys (Bray, 1969), and tryptophan (Russell and Harms, 1999). Recently, Bregendahl et al. (2008) and Silva et al. (2015) also used broken line regression to determine several AA requirements. The QP max regression is another and more subjective approach to determining nutrient requirements. Pilbrow and Morris (1974), Nathanael and Sell (1980), and Cardoso et al. (2014) are examples of the studies in which QP max was used in order to determine AA requirement of laying hens. Since broken line regression may underestimate the requirement and QP max may overestimate the requirement, the intercept of the broken line and the QP max methods can be used to determine an intermediate requirement (Baker, 2002). It was the objective of this research to determine the DLYS requirement of laying hens using increasing CP diets and the broken line, QP max and the intercept of the broken line and QP regression methods for comparison.

MATERIALS AND METHODS

All animal care procedures were approved by the university institutional animal care and use committee (IACUC). Eight layer rations were formulated using ideal AA ratios and analyzed nutrient values for all major ingredients in order to meet all nutrient requirements except Lys (Table 3.1 and Table 3.2). A basal (Diet 1) and summit (Diet 7) diet were formulated to contain

0.565% and 0.980% DLYS, 13.8% CP and 21.7% CP, respectively. Diets 2 to 6 were blended forms of the basal and summit diets in order to achieve an increasing DLYS level of 0.565 to 0.980% with respective CP levels ranging from 15.1 to 20.3%. Diets 1 to 7 in Table 3.1 were fed from 0 to 11 weeks of the trial. Diet 8 was an industry control diet for this experiment. It was formulated to contain 0.807% DLYS and had 18.6% CP. Diets were formulated based on the ideal protein concept to maintain the desired digestible AA: DLYS ratios. For the Diet 8 industry control, the ratios (%) were 73, 90, 87, 78, and 22 and 127 for Thr, Met+Cys, Val, Ile, Trp, and Arg, respectively. For the increasing CP diets 1 to 7, the ratios were increased by two percentage units in attempt that DLYS would be the first limiting AA. Thus, the digestible AA: DLYS ratios were 75, 92, 89, 80, 24, and 129, respectively. On Week 12 of the trial, the diets were changed to reduce the digestible Lys levels so that greater differences in responses to production parameters could be obtained. Again, a basal (Diet 1) and summit (Diet 7) diet were formulated to contain 0.468% and 0.845% DLYS and yielded 12.24% CP and 19.26% CP, respectively. Diets 2 to 6 were blended forms of the basal and summit diet in order to achieve an increasing DLYS level from 0.468% to 0.845% and an increasing CP level from 12.24% to 19.26%. Diet 8, an industry control, was formulated to contain 0.688% DLYS and 15.28% CP. The digestible AA: DLYS ratios were maintained at the same levels described above.

A total of 896 Lohmann LS Lite caged layers was allotted to the 8 dietary treatments, each treatment had 8 replications of 14 hens. Each traditional raised wire cage measured 17 inches high x 23 inches wide x 22 inches deep with 7 hens per cage. Each replication consisted of two adjacent cages of 7 hens. Laying hens were maintained on a 16h lighting program. Feed and water were provided *ad libitum*, and hens were fed twice daily in order to reduce feed wastage. Water was provided by nipple waters, with two nipple waters per cage. At 22 weeks of age, hens were weighed and allotted to treatments so that the mean body weight was similar for all treatments.

Eggs produced from the laying hens were counted and collected each day. Hen-day egg production were calculated every 2 weeks and corrected for mortality. Feed consumption was calculated every two weeks (g/hen/d) and was also adjusted for mortality. Mortality was recorded daily during the 25 week trial period. Feed efficiency was determined every four weeks based on egg weight (g/egg) and the amount of feed intake (g/feed).

Haugh units, egg weight, egg specific gravity, egg solids and egg grades were measured every four weeks. Eight eggs were randomly selected from each replicate group of 14 hens in order to evaluate Haugh units using the EggAnalyzer® (Orka Food Technology, Bountiful, Utah). Egg solid contents were evaluated by weighing the yolk and albumen of five eggs per replicate group and homogenizing them using a small hand held blender. Then, 3mL of both the yolk and albumen were weighed before and after drying the samples for 5 minutes in order to determine the amount of liquid and dry content of the egg. All eggs laid in a 24 hour period for each replicate group were collected for egg size grading using the Aquamagic Egg Processing Equipment® (National Poultry Equipment, Renton, Washington). Egg mass (g of egg produced per hen per d) was calculated by multiplying hen-day egg production times mean egg weight (g/egg). All eggs laid in a 24 hour-period were collected from each replicate group and measured for specific gravity every four weeks using the flotation method with NaCl solutions varying in specific gravity from 1.056 to 1.104 g/cm^3 in 0.004 increments.

The experimental design was a completely randomized design. Data were analyzed by ANOVA using SAS (SAS, 2010). Differences among treatment means ($P< 0.05$) were detected using Fisher's Protected Least Significant Difference test ($P< 0.05$). The DLYS requirement was estimated from egg production, egg mass and feed efficiency using broken line, QP max, and the intercept of the broken line and QP regression methods. Broken line regression equation described by Robbins et al. (2006) was used to determine the breakpoint of the broken line. The QP max and the intercept of the broken line and QP regression methods of Baker et al. (2002) were used in order to compare the three regression methods to each other. Egg weight, feed efficiency and egg mass production responses were analyzed from Week 6 to the end of the trial in order to determine DLYS requirements because differences among treatments generally and consistently first appeared at 6 weeks. However, egg production responses were analyzed only from Week 12 to the end of the trial because treatment effects were not observed until 12 weeks.

RESULTS

Hens fed the diet containing a mean DLYS of 0.517% DLYS and 13.0% CP had significantly lower egg production than hens fed the other diets starting at Week 12 (Table 3.3). Feed consumption (g/hen/d) was also significantly lower for the diet with lowest level of DLYS and CP starting at Week 12 (Table 3.4). There were no significant differences among diets containing 0.582% DLYS or greater. Laying hens fed diets containing the two lowest levels of DLYS and CP (0.517 to 0.582% DLYS and 13.0 to 14.3% CP) had significantly lower egg weights (Table 3.5) compared to diets containing higher levels of DLYS and CP (0.648 to 0.913% DLYS and 15.5 to 20.5% CP). Egg mass (hen-day egg production times mean egg weight) was significantly lower for Diets 1 and 2, which contained 0.517 to 0.582% DLYS and 13.0 to 14.3% CP, than the other diets (Table 3.6). Feed efficiency during the 24 week trial was generally higher for laying hens fed diets containing 0.648 to 0.913% DLYS and 15.5 to 20.5% CP than for hens fed the other two diets (Table 3.7).

Final body weight (Table 3.8) of the hens was significantly lower for diets containing the two lowest levels of DLYS and CP. Egg specific gravity and Haugh units were significantly higher for hens fed diets containing 0.517% to 0.582% DLYS and generally decreased as the DLYS and CP level increased (Table 3.8). There were no differences ($P > 0.05$) in mortality and culled hens among the treatments.

Egg grade out (%) at Week 10 of the trial showed that diets containing the two lowest levels of DLYS and CP yielded significantly more medium and small eggs, while diets with higher amounts of DLYS and CP yielded significantly more extratolarge and large eggs (Table 3.9). Similar egg grade out data were observed at Week 24 (Table 3.10). Egg yolk percentage, yolk percentage solids, white percentage solids and total percentage solids at Week 24 of the trial showed no significant differences among the dietary treatments (Table 3.11). The same results were obtained for these parameters when measured at the other 4 week periods (data not shown).

As mentioned earlier, DLYS requirements were estimated using the broken line, QP max and the intercept of the broken line and QP methods. Broken line regression analysis for egg production during Weeks 12 to 24 yielded a DLYS requirement of 528 mg/hen/d while the QP max regression analysis estimated a higher DLYS requirement of 727 mg/hen/d (Table 3.12). The intercept of the broken line and QP methods determined an intermediate DLYS requirement of 620 mg/hen/d. For egg mass, broken line regression yielded a DLYS requirement of 655 mg/hen/d, while QP max estimated the DLYS requirement at 817 mg/hen/d, and the intercept of the broken line and QP methods determined 706 mg/hen/d as the DLYS requirement. Broken line and QP max regression analysis yielded a DLYS requirement of 690 and 886 mg/hen/d for feed efficiency, respectively. The intercept of the broken line and QP methods yielded an intermediate DLYS requirement of 778 mg/hen/d.

DISCUSSION

For most of the production parameters, a similar trend that diets containing the lowest amounts of DLYS and CP yielded lower egg weight, final body weight, egg size grades, egg production, egg mass, and feed efficiency. This is likely explained by a deficiency in DLYS and/or CP in diets. Most of the response was probably due to a DLYS deficiency because the diets were formulated based on digestible AA: DLYS ratios that were designed to make DLYS first limiting. Specific gravity and Haugh units were significantly higher in diets with lower DLYS and CP compared to diets with higher levels of DLYS and CP. This may have been due to the smaller egg size since smaller eggs often have thicker egg shells and higher interior egg quality.

In order to estimate a DLYS requirement, three regression methods were used, broken line regression, QP max regression, and the intercept of the broken line and QP methods. Several researchers have used different regression methods in order to determine a Lys requirement. When egg production was the performance parameter, early papers from Bray (1969) and Gleaves and Dewan (1970) yielded reasonably similar total Lys requirements of 522 and 585 mg/hen/d, respectively. These total Lys requirement estimates agreed well with the DLYS requirement that was observed in the current study which was 528 mg/hen/d based on broken line regression. However, a higher Lys requirement was observed when QP max regression and the intercept of the broken line and QP methods were used. This was expected since QP max may overestimate the requirement, while broken line regression may underestimate the requirement (Baker, 2002). Pilbrow and Morris (1974) estimated a total Lys requirement of 820 to 920 mg/hen/d for eight different stocks of laying hens, which was based on an equation to describe the relationship between egg production, Lys intake and body weight in order to get a sigmoid response curve. This Lys requirement was much higher than earlier data suggest and was higher than the DLYS requirement determined by all the regression methods that were utilized in the current study. For corn based diets, a total Lys requirement was determined to be 660 mg/hen/d based on hen-day egg production (Sell and Johnson, 1974) and this requirement agreed well with Latshaw (1976) where a total Lys requirement was estimated at 650 mg/hen/d based on optimal egg production results. This requirement agreed well with the DLYS requirement yielded from the intercept of the broken line and QP method which was 620 mg/hen/d based on egg production results. However, it's important to note that in earlier studies the Lys requirement was determined as total Lys intake from the diets and not DLYS intake as was done in the current study. The DLYS requirement should be 10 to 15% lower than the total Lys requirement based on the expected digestibility of Lys in corn-soybean meal diets (NRC, 1994).

Using egg mass as the performance parameter, Bregendahl (2008) conducted research in White Leghorn-type laying hens at weeks 28 to 34 of age and determined a DLYS requirement of 693 mg/hen/d using broken line regression. This requirement agrees reasonably well with the broken line DLYS requirement that we observed for egg mass which was 655 mg/hen/d. Schutte and Smink (1998) determined a DLYS requirement based on egg mass response in corn based diets of 720 mg/hen/d for 24 to 36 week old White Leghorn strain hens. This DLYS requirement was estimated by taking 90% of the maximum response of the QP. Comparatively, the DLYS requirement estimated based on egg mass was 817 mg/hen/d for the current trial. If we calculate the DLYS requirement at 90% of the QP max response in the current study, a DLYS requirement of 735 mg/hen/d can be determined. This value agrees well with the Schutte and Smink (1998) estimate of 720 mg/hen/d and also is similar to the requirement yielded from the intercept of the broken line and QP methods in the current study, which was 706 mg/hen/d. This latter value is also in excellent agreement with the mean intercept of the broken line and QP max DLYS requirement of 703 mg/hen/d for Dekalb White hens from 37 to 48 weeks reported recently by Silva et al. (2015). The DLYS requirements estimated from the broken line regression, QP max and the intercept of the broken line and QP for egg mass are also shown in Figure 3.1.

When using feed efficiency as the performance parameter, Bregendahl (2008) estimated a DLYS requirement based on feed efficiency of 693 mg/hen/d based on broken line regression. Interestingly, the broken line regression estimate for feed efficiency determined in the current study was almost identical at 690 mg/hen/d. Although, as mentioned above, the intercept of the broken line and QP DLYS requirement determined herein for egg mass was in excellent agreement with the recent study by Silva et al. (2015); however the two studies are not in agreement for feed efficiency. When using the broken line and intercept of the broken line and QP methods, the DLYS requirements reported by Silva et al. (2015) are much lower than those determined herein. The reason why the two studies would be in excellent agreement for egg mass but widely different for feed efficiency is not known.

Comparing the three production parameters used in the current study, the DLYS requirement for egg mass and feed efficiency was substantially higher than the requirement based on egg production. The difference may have been primarily associated with egg weight response to dietary DLYS levels. Egg weight was affected earlier than egg production (at 2 weeks vs. 12 weeks) by DLYS level and egg weight is included in egg mass and feed efficiency calculations whereas it is not included in the egg production calculation.

When comparing the three regression methods, the broken line regression method estimated DLYS requirements that were lower than the QP max regression and intercept of the broken and QP for all three production parameters evaluated. The highest DLYS requirements were estimated using the QP max regression. Consistently, the intercept of the broken line and QP methods yielded a requirement that was intermediate between the broken line and QP max regression. This was expected since an earlier study showed that the broken line and QP max regression methods may underestimate or overestimate the requirement, respectively, and that the intercept of the broken line and QP methods would provide an objective intermediate value that may be more economically realistic (Baker, 2002). Similar results were recently reported by Silva et al. (2015).

The intercept of the broken linen and QP DLYS requirement for egg mass (706 mg/hen/d) determined herein is substantially higher than the NRC (1994) requirement. The latter publication lists 690 mg/hen/d as the total LYS requirement for laying hens. With the digestibility of LYS in corn – soybean meal diets being approximately 88% (NRC, 1994), the total LYS requirement value calculates to be a DLYS requirement of only 607 mg/hen/d. The higher DLYS requirement in the current study is probably due mainly to genetic differences between the hens used herein and those used in the much older studies on which the NRC (1994) requirement is based. It is interesting, however, that the intercept of the broken line and QP DLYS requirement for egg mass of 706 mg/hen/d determined herein for 23 to 46 week old hens is almost identical to the DLYS value of 710 mg/hen/d recommended by the breeder for Lohmann LS Lite hens from 29 to 45 weeks (Lohmann Tierzucht, GMBH, 2014), indicating very good agreement between the results of the current study and the breeder recommendation.

In summary, several regression methods can be used to determine a DLYS requirement for laying hens. The current study showed that for 22 to 47 week old Lohmann hens, the intercept of the broken line and QP methods yielded DLYS requirement estimates of 706, 620, 730 mg/hen/d based on responses from egg mass, egg production and feed efficiency, respectively. These DLYS requirements may be more economically advantageous than the requirements yielded from the broken line and QP max regression.

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TABLES AND FIGURE

TABLE 3.1. Ingredient and nutrient compositions of the experimental diets provided to laying hens for 0 to 12 weeks (23 to 34 weeks of age).

	Dietary treatments							
Ingredients	1	2	3	4	5	6	7	8
	(%)							
Corn	66.39	62.49	58.59	54.67	50.77	46.86	42.95	52.67
Soybean meal	11.16	14.38	17.60	20.82	24.05	27.27	30.49	22.22
DDGS[1]	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
MBM[1]	1.46	1.58	1.71	1.83	1.95	2.07	2.20	2.55
Soy oil	0.03	0.61	1.19	1.78	2.36	2.95	3.53	2.02
Limestone	9.62	9.59	9.57	9.55	9.53	9.50	9.48	9.47
Dicalcium phosphate	0.62	0.56	0.50	0.44	0.37	0.31	0.25	0.25
Salt	0.29	0.29	0.29	0.29	0.29	0.29	0.29	0.28
Vitamin mix[2]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Mineral mix[3]	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04
L-Lys HCl	0.08	0.07	0.06	0.05	0.04	0.03	0.03	0.04
DL-Met	0.12	0.16	0.20	0.25	0.29	0.33	0.37	0.24
L-Thr	0.01	0.02	0.03	0.04	0.06	0.07	0.08	0.03
L-Val		0.02	0.03	0.05	0.06	0.08	0.09	0.03
L-Ile	0.02	0.03	0.03	0.04	0.04	0.05	0.05	0.02
L-Trp	0.02	0.02	0.02	0.01	0.01	0.01	0.01	
Choline chloride	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
NSP Enzyme[4]	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Phytase[4]	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Calculated composition[1]								
ME, kcal/kg	2900	2900	2900	2900	2900	2900	2900	2900
Crude protein, %	13.78	15.09	16.40	17.72	19.03	20.34	21.66	18.55
Calcium, %	4.10	4.10	4.10	4.10	4.10	4.10	4.10	4.10
Available phosphorus, %	0.44	0.44	0.44	0.44	0.44	0.44	0.44	0.44
Sodium, %	0.18	0.18	0.18	0.18	0.18	0.18	0.19	0.18
Digestible Lys, %	0.565	0.634	0.703	0.733	0.842	0.911	0.980	0.807
Digestible Thr, %	0.424	0.476	0.528	0.580	0.631	0.683	0.735	0.589
Digestible Met + Cys, %	0.520	0.584	0.647	0.711	0.775	0.838	0.902	0.726

[1]Calculated composition based on protein and amino acid analysis of the corn, soybean meal, distiller's dried grains with solubles (DDGS) and meat and bone meal (MBM).

[2]Provided the following per kg of diet: 9,370 IU of vitamin A, 3,031 IU of vitamin D_3, 28 IU of vitamin E, 0.013 mg of vitamin B_{12}. 0.088 mg of biotin, 1.9 mg of menadione, 1.9 mg of thiamine, 7.7 mg of riboflavin, 12.1 mg of dtopantothenic acid, 3.1 mg of pyridoxine, 49.6 mg of niacin, .99 mg of folic acid.

[3]Provided the following per kg of diet: 120 mg of manganese, 99 mg of zinc, 40 mg of iron, 25 mg of magnesium, 10 mg of copper, 1 mg of iodine, .3 mg of selenium.

TABLE 3.1. (Cont.)

[4]NSP Enzyme was Econase, Cincinnati, Ohio. Phytase was provided by AB Vista, Marlborough Wiltshire, United Kingdom and was assumed to release 0.1% for available P, 0.05% calcium and 0.02% sodium.

TABLE 3.2. Ingredient and nutrient compositions of the experimental diets provided to laying hens for 13 to 24 weeks (35 to 46 weeks of age).

	Dietary treatments							
Ingredients	1	2	3	4	5	6	7	8
	(%)							
Corn	67.59	64.28	60.97	57.66	54.36	51.05	47.74	60.94
Soybean meal	7.41	10.04	12.66	15.29	17.91	20.53	23.16	14.19
DDGS[1]	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50
MBM[1]		0.34	0.68	1.03	1.37	1.71	2.05	0.39
Soy oil	0.03	0.49	0.94	1.40	1.85	2.30	2.76	0.50
Limestone	9.83	9.78	9.74	9.70	9.65	9.61	9.56	9.75
Dicalcium phosphate	0.89	0.78	0.68	0.57	0.46	0.36	0.25	0.72
Salt	0.32	0.31	0.30	0.30	0.29	0.28	0.27	0.29
Vitamin mix[2]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Mineral mix[3]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
L-Lys HCl	0.06	0.05	0.05	0.05	0.04	0.04	0.03	0.13
DL-Met	0.06	0.10	0.14	0.18	0.21	0.25	0.29	0.20
L-Thr		0.01	0.01	0.02	0.03	0.03	0.04	0.04
L-Val				0.01	0.01	0.01	0.01	
L-Ile		0.01	0.01	0.02	0.03	0.03	0.04	0.04
L-Trp	0.10	0.09	0.07	0.06	0.05	0.03	0.02	0.01
Choline chloride	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Filler[4]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	
Rovabio Max[5]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Calculated composition[1]								
ME, kcal/kg	2900	2900	2900	2900	2900	2900	2900	2900
Crude protein, %	12.24	13.41	14.58	15.75	16.92	18.09	19.26	15.28
Calcium, %	4.10	4.10	4.10	4.10	4.10	4.10	4.10	4.10
Available phosphorus, %	0.44	0.44	0.44	0.44	0.44	0.44	0.44	0.44
Sodium, %	0.18	0.18	0.18	0.18	0.18	0.18	0.19	0.18
Digestible Lys, %	0.468	0.531	0.594	0.657	0.719	0.782	0.845	0.688
Digestible Thr, %	0.368	0.412	0.457	0.501	0.545	0.590	0.634	0.502
Digestible Met + Cys, %	0.431	0.489	0.546	0.604	0.662	0.719	0.777	0.619

[1]Calculated composition based on protein and amino acid analysis of the corn, soybean meal distiller's dried grains with solubles (DDGS) and meat and bone meal (MBM).

[2]Provided the following per kg of diet: 9,370 IU of vitamin A, 3,031 IU of vitamin D_3, 28 IU of vitamin E, 0.013 mg of vitamin B_{12}. 0.088 mg of biotin, 1.9 mg of menadione, 1.9 mg of thiamine, 7.7 mg of riboflavin, 12.1 mg of dtopantothenic acid, 3.1 mg of pyridoxine, 49.6 mg of niacin, .99 mg of folic acid.

[3]Provided the following per kg of diet: 120 mg of manganese, 99 mg of zinc, 40 mg of iron, 25 mg of magnesium, 10 mg of copper, 1 mg of iodine, .3 mg of selenium.

[4]Filler=sand

TABLE 3.2. (Cont.)

[5]Rovabio Max was provided by Adisseo, Alpharetta, Georgia and it was assumed to release 0.11% for available P, 0.06% calcium and 0.03% sodium.

TABLE 3.3. Effect of increasing digestible lysine and CP on hen-day egg production.

Diet	DLYS[1] (%)	DLYS[2] intake (mg/hen/d)	Weeks 2	6	10	12	14	18	22	24
			(%)							
1	0.517	389	97.5	96.8	95.7	94.2[b]	84.8[b]	76.7[b]	68.0[c]	66.5[c]
2	0.582	521	97.1	97.5	96.7	96.5[a]	94.3[a]	93.5[a]	89.3[b]	90.2[b]
3	0.648	587	98.1	96.7	96.6	97.4[a]	96.6[a]	95.0[a]	94.2[a]	94.3[ab]
4	0.695	658	96.7	97.5	96.4	97.2[a]	96.5[a]	95.9[a]	94.8[a]	95.2[a]
5	0.781	722	97.0	98.0	97.9	97.1[a]	96.2[a]	96.0[a]	94.8[a]	95.1[a]
6	0.847	790	96.3	97.4	97.8	97.4[a]	96.1[a]	96.5[a]	94.4[a]	96.2[a]
7	0.913	846	96.1	98.1	97.6	97.7[a]	97.2[a]	95.5[a]	94.7[a]	95.0[a]
8	0.748	684	97.9	96.3	97.3	97.5[a]	96.7[a]	94.2[a]	94.2[a]	93.8[ab]
Pooled SEM			0.6	0.7	0.7	0.7	0.9	1.2	1.5	1.5

[1]DLYS= Digestible Lys, mean of the diets fed during Weeks 0 to 12 and 13 to 24 (Tables 3.1 and 3.2)

[2]Digestible Lys intake for Weeks 12 to 24 which was included in the regression analyses to estimate DLYS requirements.

TABLE 3.4. Effect of increasing digestible lysine and CP on feed consumption.

			Weeks							
Diet	DLYS[1] (%)	DLYS[2] intake (mg/hen/d)	2	6	10	12	14	18	22	24
			(g/hen/d)							
1	0.517	442	94.5	92.1	97.4	90.7[b]	89.1[b]	84.6[b]	76.5[b]	75.7[b]
2	0.582	558	95.1	94.7	100.1	97.6[a]	98.7[a]	99.6[a]	97.1[a]	96.5[a]
3	0.648	623	93.4	93.9	100.1	98.5[a]	98.1[a]	99.6[a]	98.8[a]	99.2[a]
4	0.695	697	94.2	95.1	100.8	98.9[a]	98.6[a]	101.9[a]	99.3[a]	101.1[a]
5	0.781	762	92.5	95.7	101.8	99.7[a]	99.0[a]	102.3[a]	99.0[a]	102.3[a]
6	0.847	831	93.6	95.6	101.2	99.4[a]	98.9[a]	102.4[a]	101.0[a]	103.2[a]
7	0.913	888	92.1	94.8	100.3	98.9[a]	98.6[a]	101.1[a]	100.8[a]	101.2[a]
8	0.748	726	94.4	95.2	101.1	99.0[a]	98.3[a]	100.7[a]	98.4[a]	100.5[a]
Pooled SEM			0.9	0.8	1.3	1.5	1.7	2.5	3.2	2.9

[1]DLYS= Digestible Lys, mean of the diets fed during Weeks 0 to 12 and 13 to 24 (Tables 3.1 and 3.2)

[2]Digestible Lys intake for Weeks 6 to 24 which was included in the regression analyses to estimate DLYS requirements.

TABLE 3.5. Effect of increasing digestible lysine and CP on egg weight.

Diet	DLYS[1] (%)	DLYS[2] intake (mg/hen/d)	Weeks 2	6	10	14	18	22	24
			(g/egg)						
1	0.517	442	51.5[b]	53.2[d]	55.3[d]	53.3[d]	50.8[e]	50.6[d]	52.2[d]
2	0.582	558	52.3[b]	55.1[cd]	56.6[c]	55.6[c]	54.7[d]	56.9[c]	56.1[c]
3	0.648	623	53.6[a]	56.1[bc]	58.2[ab]	57.1[bc]	57.1[c]	57.7[bc]	58.6[b]
4	0.695	697	53.8[a]	56.6[ab]	58.9[ab]	58.2[ab]	59.0[ab]	59.1[ab]	59.8[ab]
5	0.781	762	54.6[a]	57.3[ab]	59.0[ab]	59.2[a]	59.6[ab]	60.0[a]	60.0[ab]
6	0.847	831	53.8[a]	57.2[ab]	59.3[a]	59.2[a]	59.5[ab]	60.1[a]	60.0[ab]
7	0.913	888	53.9[a]	57.5[a]	59.6[a]	59.5[a]	60.6[a]	60.7[a]	60.80[a]
8	0.748	726	54.6[a]	57.2[ab]	59.3[a]	58.1[ab]	58.1[bc]	59.0[ab]	58.9[ab]
Pooled SEM			0.48	0.41	0.49	0.52	0.58	0.69	0.72

[1]DLYS = Digestible Lys, mean of the diets fed during Weeks 0 to 12 and 13 to 24 (Tables 3.1 and 3.2).
[2]Digestible Lys intake for Weeks 6 to 24 which was included in the regression analyses to estimate DLYS requirements

TABLE 3.6. Effect of increasing digestible lysine and CP on egg mass.

Diet	DLYS[1] (%)	DLYS[2] intake (mg/hen/d)	Weeks 2	6	10	14	18	22	24
			(g egg/hen/d)						
1	0.517	442	50.3^{c}	51.4^{d}	52.8^{d}	47.7^{d}	40.0^{d}	35.7^{c}	34.8^{c}
2	0.582	558	50.8^{bc}	53.7^{c}	54.9^{c}	53.1^{c}	51.1^{c}	50.7^{b}	50.6^{b}
3	0.648	623	52.6^{a}	54.2^{bc}	56.2^{bc}	55.4^{b}	54.2^{b}	54.8^{ab}	55.3^{a}
4	0.695	697	52.0^{ab}	55.2^{ab}	57.1^{ab}	56.4^{ab}	56.6^{ab}	56.2^{a}	56.9^{a}
5	0.781	762	53.0^{a}	56.1^{a}	57.6^{ab}	57.3^{ab}	57.2^{a}	57.3^{a}	57.1^{a}
6	0.847	831	51.8^{abc}	55.8^{a}	58.0^{ab}	57.3^{ab}	57.6^{a}	57.6^{a}	57.7^{a}
7	0.913	888	51.8^{abc}	56.4^{a}	58.2^{a}	58.0^{a}	57.7^{a}	56.9^{a}	57.9^{a}
8	0.748	726	52.5^{a}	55.1^{ab}	57.8^{ab}	56.4^{ab}	55.2^{ab}	55.9^{a}	55.2^{a}
Pooled SEM			0.57	0.52	0.63	0.74	0.92	1.20	1.27

[1]DLYS = Digestible Lys, mean of the diets fed during Weeks 0 to 12 and 13 to 24 (Tables 3.1 and 3.2).

[2]Digestible Lys intake for Weeks 6 to 24 which was included in the regression analyses to estimate DLYS requirements.

TABLE 3.7. Effect of increasing digestible lysine and CP on feed efficiency.

Diet	DLYS[1] (%)	DLYS[2] intake (mg/hen/d)	Weeks 2	6	10	14	18	22	24
			(g egg/ g feed)						
1	0.517	442	0.532[c]	0.558[d]	0.552[c]	0.525[c]	0.479[d]	0.463[c]	0.460[c]
2	0.582	558	0.534[c]	0.567[cd]	0.553[c]	0.539[c]	0.514[c]	0.524[b]	0.525[b]
3	0.648	623	0.563[ab]	0.578[bc]	0.567[bc]	0.564[b]	0.544[b]	0.552[ab]	0.558[a]
4	0.695	697	0.553[b]	0.580[abc]	0.572[ab]	0.570[b]	0.557[ab]	0.568[a]	0.564[a]
5	0.781	762	0.573[a]	0.586[ab]	0.575[ab]	0.577[ab]	0.560[ab]	0.582[a]	0.559[a]
6	0.847	831	0.553[b]	0.583[ab]	0.578[ab]	0.578[ab]	0.563[ab]	0.570[a]	0.560[a]
7	0.913	888	0.563[ab]	0.595[a]	0.586[a]	0.587[a]	0.571[a]	0.567[a]	0.572[a]
8	0.748	726	0.567[ab]	0.579[bc]	0.576[ab]	0.570[b]	0.550[ab]	0.571[a]	0.550[b]
Pooled SEM			0.0061	0.0053	0.0059	0.0055	0.0087	0.0121	0.0077

[1]DLYS = Digestible Lys, mean of the diets fed during Weeks 0 to 12 and 13 to 24 (Tables 3.1 and 3.2).
[2]Digestible Lys intake for Weeks 6 to 24 which was included in the regression analyses to estimate DLYS requirements.

TABLE 3.8. Effects of increasing digestible lysine and CP on final body weight, egg specific gravity, and haugh units at 24 week of experiment.

Diet	DLYS[1] (%)	DLYS intake[2] (mg/hen/d)	Final body weight (g/hen)	Egg specific gravity (g/cm^3)	Haugh units
1	0.517	442	1316[d]	1.0892[a]	80.19[a]
2	0.582	558	1498[c]	1.0853[b]	78.07[abc]
3	0.648	623	1576[ab]	1.0830[c]	78.96[ab]
4	0.695	697	1637[a]	1.0828[c]	76.68[bcd]
5	0.781	762	1650[a]	1.0822[c]	77.31[bcd]
6	0.847	831	1621[ab]	1.0826[c]	75.36[d]
7	0.913	888	1646[a]	1.0833[c]	76.54[cd]
8	0.748	726	1596[ab]	1.0833[c]	76.62[cd]
Pooled SEM			26	0.0017	0.83

[1]DLYS = Digestible Lys, mean of the diets fed during Weeks 0 to 12 and 13 to 24 (Table 3.1 and 3.2)

[2]Digestible Lys intake for Weeks 6 to 24 which was included in the regression analyses to estimate DLYS requirements.

TABLE 3.9. Effects of increasing digestible lysine and CP on percent egg grades at Week 10.

Diet	DLYS[1] (%)	DLYS intake[2] (mg/hen/d)	Jumbo (%)	Extra large (%)	Large (%)	Medium (%)	Small (%)
1	0.517	442	0	2.75[d]	24.36[d]	63.68[a]	9.21[a]
2	0.582	558	0	7.42[cd]	36.64[bcd]	52.42[ab]	3.51[b]
3	0.648	623	0	13.84[abc]	45.09[abc]	39.14[bc]	1.93[bc]
4	0.695	697	0.89	14.77[abc]	50.65[ab]	31.67[c]	2.00[bc]
5	0.781	762	0.89	20.19[ab]	44.23[abc]	34.68[c]	0[c]
6	0.847	831	0	22.11[a]	51.44[a]	24.65[c]	1.78[bc]
7	0.913	888	0.89	20.54[a]	44.64[abc]	33.93[c]	0[c]
8	0.748	726	0	17.04[abc]	53.39[a]	29.56[c]	0[c]
Pooled SEM			0.57	3.60	5.24	5.74	1.23

[1]DLYS = Digestible Lys, mean of the diets fed during Weeks 0 to 12 and 13 to 24 (Tables 3.1 and 3.2).

[2]Digestible Lys intake for Weeks 6 to 24 which was included in the regression analyses to estimate DLYS requirements.

TABLE 3.10. Effects of increasing digestible lysine and CP on percent egg grades at Week 24.

Diet	DLYS (%)	DLYS intake (mg/hen/d)	Jumbo (%)	Extra large (%)	Large (%)	Medium (%)	Small (%)	PeeWee (%)
1	0.517	442	0	0.89[e]	5.06[d]	70.37[a]	21.58[a]	2.08
2	0.582	558	0	7.71[de]	28.65[bc]	58.30[ab]	5.33[b]	0
3	0.648	623	0	21.61[bcd]	36.55[abc]	40.95[bc]	0.89[b]	0
4	0.695	697	3.77	23.01[bc]	39.99[ab]	31.29[c]	1.92[b]	0
5	0.781	762	0.89	56.57[a]	47.22[a]	23.31[c]	2.00[b]	0
6	0.847	831	0	24.72[bc]	48.37[a]	26.00[c]	0.89[b]	0
7	0.913	888	1.13	34.74[b]	40.98[ab]	22.18[c]	0.96[b]	0
8	0.748	726	1.04	11.82[cde]	50.54[a]	35.70[c]	0.89[b]	0
Pooled SEM			0.82	4.74	5.24	6.58	2.36	0.54

[1]DLYS = Digestible Lys, mean of the diets fed during Weeks 0 to 12 and 13 to 24 (Tables 3.1 and 3.2).
[2]Digestible Lys intake for Weeks 6 to 24 which was included in the regression analyses to estimate DLYS requirements.

TABLE 3.11. Effect of increasing digestible lysine and CP on yolk percentage, yolk percentage solids, white percentage solids and total percentage solids at Week 24.

Diet	DLYS (%)	DLYS Intake (mg/hen/d)	Yolk %	Yolk % Solids	White % Solids	Total % Solids
1	0.517	442	32.43	50.32	11.36	23.57
2	0.582	558	33.32	50.33	11.17	23.81
3	0.648	623	33.09	50.28	11.24	23.73
4	0.695	697	32.54	50.55	11.12	23.57
5	0.781	762	32.99	50.34	11.42	23.90
6	0.847	831	32.44	50.07	11.48	23.77
7	0.913	888	32.52	50.24	11.69	23.83
8	0.748	726	33.20	50.55	11.22	23.93
Pooled SEM			0.37	0.21	0.13	0.17

[1]DLYS = Digestible Lys, mean of the diets fed during Weeks 0 to 12 and 13 to 24 (Tables 3.1 and 3.2).
[2]Digestible Lys intake for Weeks 6 to 24 which was included in the regression analyses to estimate DLYS requirements.

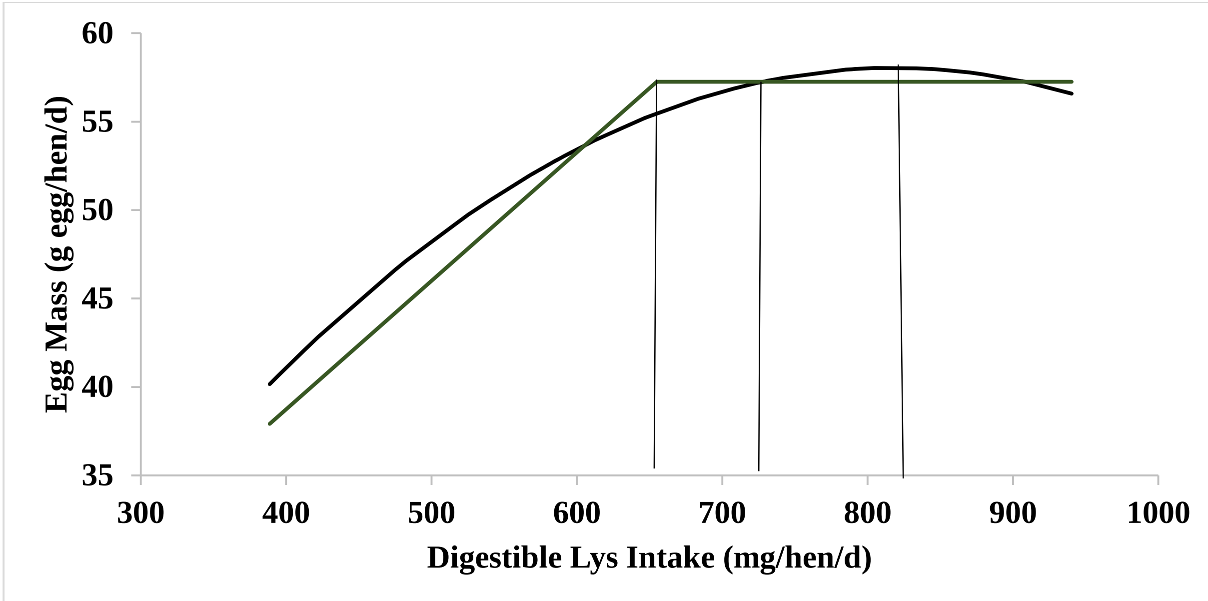
TABLE 3.12. Summary of the requirement methods and the digestible lysine requirements estimated using the three different regression methods.

Regression method	Egg production	Egg mass	Feed efficiency
		(mg/hen/d)	
Broken Line	528 (.96)[1]	655 (.86)	690 (.52)
QP max[2]	727 (.92)	817 (.85)	886 (.51)
Intercept of the broken line and QP	620	706	778

[1]Values in parenthesis are R^2 values for the regression model

[2]QP max= the maximum of the quadratic polynomial.

FIGURE 3.1. Broken line, quadratic polynomial max, the intercept of the broken line and quadratic polynomial for egg mass for the inreasing protein diets.



CHAPTER 4: DETERMINING THE DIGESTIBLE LYSINE REQUIREMENT OF 22 TO 47 WEEK-OLD LOHMANN LAYING HENS USING A CONSTANT CRUDE PROTEIN TITRATION METHOD

ABSTRACT

Starting at 22 weeks of age, a large laying hen trial was conducted to determine the digestible Lys (DLYS) requirement of laying hens by using a constant crude protein (CP) titration method. Eight hundred and ninety six Lohmann LS Lite caged layers were allotted to 8 dietary treatments with 8 replications each of 14 hens from 22 to 47 weeks of age. The first 7 experimental diets contained DLYS levels increasing from 0.565 to 0.980% with respective protein levels increasing slightly from 15.9 to 16.5% due to Lys supplementation. Dietary Treatment 8 was an industry control diet which was calculated to contain 18.6% CP and 0.807% DLYS. The DLYS levels were decreased to 0.468 to 0.845% at Week 12 to obtain greater differences in production parameters among diets. Increasing DLYS levels had a significant effect ($P < 0.05$) on egg production, egg weight, egg mass, and feed efficiency during Weeks 14 to 24 of the trial. DLYS levels had little or no effect on egg component measurements such as percentage of yolk, white and solids. Three types of regression methods were used to determine the DLYS requirement: Broken line regression, maximum of the quadratic polynomial (QP max) regression, and the intercept of the broken line and QP regressions. Broken line regression yielded the lowest requirement values for egg production, egg mass, and feed efficiency and QP max yielded the highest values, with the intercept of the broken line and QP methods yielding intermediate requirement estimates. For egg mass, DLYS requirements were

approximately 703, 863, and 772 mg/hen/d for the broken line, QP max, and the intercept of the broken line and QP models, respectively.

Keywords: digestible lysine, crude protein, laying hens, broken line regression, quadratic polynomial regression

INTRODUCTION

Accurately determining the Lys requirement of laying hens is vital to achieving maximum production since Lys is used as the reference amino acid (AA) when formulating diets based on ideal protein ratios (Baker, 1997). In addition, the proper use of Lys and other AAs often leads to an increase in economic revenue (Silva et al., 2015). However, recently, there has been little research to determine the DLYS requirement in laying hens despite 15 to 40 years of genetic improvement (Bregendahl et al., 2008; Novak, 2004). Therefore, it is essential to accurately determine the Lys requirement in current strains of laying hens in order to achieve maximum production and revenue.

As discussed previously, Lys requirements have been determined by several researchers with vast differences reported among the requirement estimates. In the 1960s, Lys requirements of 494, 454, and 522 mg/hen/d were reported by Harms and Waldroup (1963), Moran et al., (1967) and Bray (1969), respectively. However, a trend for the Lys requirement to increase over a period of time is evidenced by the studies of Schutte and Sminks (1998), Bregendahl et al. (2008) and Silva et al. (2015).

Broken line regression has often been used to determine nutrient requirements since the model is relatively objective in determining an estimate. Broken line regression was commonly used in many earlier studies to determine requirements for methionine

(Bray, 1965), for Lys (Bray, 1969), and tryptophan (Russell and Harms, 1999) for laying hens. Broken line regression was also used in more recent studies of Bregendahl et al. (2008) and Silva et al. (2015). The QP max regression method is another and more subjective approach to determining nutrient requirements. Several researchers have used QP max to determine nutrient requirements over the course of several years (Pilbrow and Morris, 1974; Nathanael and Sell, 1980; Cardoso et al., 2014). However, it is suggested that broken line regression may underestimate the requirement and QP max regression may overestimate the requirement of the general animal population (Baker et al., 2002). Consequently, the intercept of the broken line and QP methods can be used to determine an intermediate requirement (Baker et al., 2002) that may have more practical application. The previous research conducted in Chapter 3 evaluated and compared all three of the regression methods for determining the DLYS requirement of a modern strain of laying hens using an increasing dietary protein approach. The objective of the current study was to determine the DLYS requirement of laying hens using constant CP diets and the broken line, QP max and the intercept of the broken line and QP regression methods for comparison to the results obtained in Chapter 3.

MATERIALS AND METHODS

All animal care procedures were approved by the university institutional animal care and use committee (IACUC). Eight layer rations were formulated using ideal AA ratios and analyzed nutrient values for all major ingredients in order to meet all nutrient requirements except Lys (Table 4.1 and Table 4.2). A basal (Diet 1) and summit (Diet 7) diet were formulated to contain 0.565% and 0.980% DLYS, respectively. Diets 2-6 were blended forms of the basal and summit diets in order to achieve an increasing DLYS level of 0.565 to 0.980% with respective CP levels ranging from 15.9 to 16.5%. Dietary CP level increased slightly due to the addition of L-Lys HCl in place of corn for Diets 1 to 7. In Table 4.1 Diets 1 to 7 were fed from Week 0- 11Week of the trial. Diet 8 was an industry control diet for this experiment. It was formulated to contain 0.807% DLYS and 18.6% CP. Diets were formulated based on the ideal protein concept to maintain the desired digestible AA: DLYS ratios. For the Diet 8 industry control, the ratios (%) were 73, 90, 87, 78, and 22 and 127 for Thr, Met+Cys, Val, Ile, Trp, and Arg, respectively. For Diet 7, the ratios were increased by two percentage units so that DLYS would be the first limiting AA, in all Diets 1 to 7. Thus, the digestible AA: DLYS ratios for Diet 7 were 75, 92, 89, 80, 24, and 129, respectively. The levels of supplemental Thr, Met+Cys, Val, Ile, Trp, and Arg were kept constant for Diets 1 to 7; consequently, as the DLYS level decreased from Diet 7 to Diet 1 the AA: DLYS ratios increased. On Week 12 of the trial, the diets were changed to reduce the digestible Lys levels so that greater differences in responses to production parameters could be obtained. Again, a basal (Diet 1) and summit (Diet 7) diet were formulated to contain 0.468% and 0.845% DLYS. Diets 2 to 6 were blended forms of the basal and summit diet in order to achieve an increasing DLYS level from 0.468% to 0.845% and a relatively constant CP level at 14 to 14.5%. Diet 8, an industry control, was formulated to contain 0.688% DLYS and 15.28% CP. The digestible AA: DLYS ratios were maintained at approximately the same levels described above.

A total of 896 Lohmann LS Lite caged layers was allotted to the 8 dietary treatments, each treatment had 8 replications of 14 hens. Each traditional raised wire cage measured 17 inches high x 23 inches wide x 22 inches deep with 7 hens per cage. Each replication consisted of two adjacent cages of 7 hens. Laying hens were maintained on a 16h lighting program. Feed and water were provided *ad libitum*, and hens were fed twice daily in order to reduce feed wastage. Water was provided by nipple waters, with two nipple waters per cage. At 22 weeks of age, hens were weighed and allotted to treatments so that the mean body weight was similar for all treatments.

Eggs produced from the laying hens were counted and collected each day. Hentoday egg production was calculated every 2 weeks and corrected for mortality. Feed consumption was calculated every two weeks (g/hen/day) and was also adjusted for mortality. Mortality was recorded daily during the 25 week trial period. Feed efficiency was determined every four weeks based on egg weight (g/egg) and the amount of feed intake (g/feed).

Haugh units, egg weight, egg specific gravity, egg solids and egg grades were measured every four weeks. Eight eggs were randomly selected from each replicate group of 14 hens in order to evaluate Haugh units using the EggAnalyzer® (Orka Food Technology, Bountiful, Utah). Egg solid contents were evaluated by weighing the yolk and albumen of five eggs per replicate group and homogenizing them using a small hand held blender. Then, 3mL of both the yolk and albumen were weighed before and after drying the samples for 5 minutes in order to determine the amount of liquid and dry content of the egg. All eggs laid in a 24 hour period for each replicate group were collected for egg size grading using the Aquamagic Egg Processing Equipment® (National Poultry Equipment, Renton, Washington). Egg mass (g of egg produced per hen per day) was calculated by multiplying hen-day egg production times mean egg weight (g/egg). All eggs laid in a 24 hour-period were collected from each replicate group and measured for specific gravity every four weeks using the flotation method with NaCl solutions varying in specific gravity from 1.056 to 1.104 g/cm^3 in 0.004 increments.

The experimental design was a completely randomized design. Data were analyzed by ANOVA using SAS (SAS, 2010). Differences among treatment means ($P< 0.05$) were detected using Fisher's Protected Least Significant Difference test ($P< 0.05$). The DLYS requirement was estimated from egg production, egg mass and feed efficiency using broken line, QP max, and the intercept of the broken line and the QP regression methods. Broken line regression equation described by Robbins et al. (2006) was used to determine the breakpoint of the broken line. The QP max and intercept of the broken line and QP regression methods of Baker et al. (2002) were used in order to compare the three regression methods to each other. Feed efficiency, egg mass, and egg production responses were analyzed from Week 14 to the end of the trial in order to determine DLYS requirements because differences among treatments generally and consistently first appeared at 14 weeks.

RESULTS

Hens fed the diet containing a mean DLYS of 0.517% DLYS had significantly lower egg production than hens fed most of the other diets starting at Week 14 (Table 4.3). Feed consumption (g/hen/d) was not significantly affected by dietary treatment (Table 4.4) except for Week 24 where it was lower for hens fed the 0.517% DLYS than hens fed 0.648% DLYS or higher. Egg weights (Table 4.5) were significantly lower for hens fed the lowest two levels of DLYS (0.517% and 0.582%) compared to hens fed 0.648 to 0.913% DLYS during most weeks in the 14 to 24 week period. Similar results were observed for egg mass (Table 4.6). As observed for egg production, egg weights, and egg mass, consistent differences among treatments for feed efficiency also were not observed until 14 weeks of age when the values for the lowest two or three DLYS treatment levels were significantly reduced compared to the other higher DLYS treatments.

Final body weight (Table 4.8) was significantly lower for the hens fed the diet containing the lowest level of DLYS than for hens on the other treatments. Egg specific gravity was significantly higher for hens fed the diet containing 0.517% DLYS compared to hens on the other dietary treatments (Table 4.8). There were no differences ($P > 0.05$) in mortality and culled hens among the treatments (data not shown).

Egg grade out data at Week 14 (Table 4.9) of the trial showed that the lowest DLYS treatment (0.517%) yielded significantly more medium and small eggs, and fewer large eggs than most of the other dietary treatments with higher DLYS levels. This same treatment effect was observed at Week 24 (Table 4.10). Egg yolk percentage, yolk percentage solids, white percentage solids and total percentage solids at Week 24 of the trial showed no significant differences among the dietary treatments (Table 4.11). The same results were obtained for these parameters when measured at the other 4 week periods (data not shown).

As previously discussed, DLYS requirements were estimated using the broken line, QP max and the intercept of the broken line and QP methods based on production responses from Week 14 to 24 of the trial. For egg production, broken line regression yielded a DLYS requirement of 686 mg/hen/d while the QP max regression analysis estimated a higher DLYS requirement of 833 mg/hen/d (Table 4.12). The intercept of the broken line and QP methods determined an intermediate DLYS requirement of 754 mg/hen/d. For egg mass, broken line regression yielded a DLYS requirement of 703 mg/hen/d, while the QP max estimated the DLYS requirement at 863 mg/hen/d, and the intercept of the broken line and QP methods determined 772 mg/hen/d as the DLYS requirement. For feed efficiency, the broken line and QP max regression analysis yielded DLYS requirements of 698 and 824 mg/hen/d, respectively. The intercept of the broken line and QP methods estimated an intermediate DLYS requirement of 737 mg/hen/d.

DISCUSSION

For most production parameters, a similar trend for diets containing the lowest one or two levels of DLYS to yield lower responses was observed. The latter effect was probably due to a deficiency in DLYS since this was the only AA or nutrient that differed among Diets 1 to 7. In contrast, the Diet 1 which contained the lowest DLYS level yielded the highest egg specific gravity. Since small eggs usually have thicker egg shells, the reduced egg size for the Diet 1 treatment may explain why egg specific gravity was significantly higher for that diet containing the lowest level of DLYS.

Broken line regression, QP max, and the intercept of the broken line and QP methods were used in order to comprehensively estimate the DLYS requirement of laying hens. Several researchers have previously estimated the Lys requirement of laying hens using different methods for egg production. A total Lys requirement for corn based diets was estimated at 660 mg/hen/d based on egg production by Sell and Johnson (1974). Similarly, a total Lys requirement based on egg production responses was determined by Latshaw (1976) at 650 mg/hen/d. For the current study, broken line regression analysis determined a DLYS requirement at 686 mg/hen/d based on egg production results which agrees well with the Sell and Johnson (1974) and Latshaw (1976) earlier studies. In addition, Pilbrow and Morris (1974) estimated a total Lys requirement of 820 to 920 mg/hen/d for eight different stocks of laying hens, which was based on an equation to describe the relationship between egg production, Lys intake and body weight in order to get a sigmoid response curve. This Lys requirement range was higher than the egg production DLYS requirements estimated using the broken line and intercept of the broken line and QP methods in the current study. However, the DLYS requirement for egg production determined by the QP max regression, herein, did fall within the range reported by Pilbrow and Morris (1974). When comparing the results of the current study to the earlier studies, it is important to comment on the difference between total and DLYS. In earlier studies, the total requirement was determined as total Lys. The DLYS requirement, such as that reported herein, should be 10 to 15% lower than the total Lys requirement in corn-soybean meal diets based on the digestibility of Lys in corn and soybean meal (NRC, 1994).

In addition to egg production, egg mass is a very important production measurement. The latter was used in an earlier study to determine the DLYS requirement in 28 to 34 week old laying hens and the requirement was estimated to be 693 mg/hen/d when using broken line regression (Bregendahl, 2008). This requirement agrees reasonably well with the broken line DLYS requirement in the current study for egg mass which was 703 mg/hen/d. Schutte and Smink (1998) determined a DLYS requirement for White Leghorn hens based on egg mass by taking 90% of the QP max. Using this procedure, a DLYS requirement of 720 mg/hen/d was determined. The QP max regression in the current study yielded a requirement of 863 mg/hen/d for maximum egg mass. If 90% of that value is calculated, a DLYS requirement of 777 mg/hen/d is estimated. The latter requirement is approximately 8% higher than Schutte and Smink (1998) estimate but agrees very well with the DLYS requirement obtained from the intercept of the broken line and QP methods in the current study which was 772 mg/hen/d. The intercept of the broken line and QP requirement value of 772 mg/hen/d is, however, somewhat higher than the mean intercept of the broken line and QP DLYS requirement (703 mg/hen/d) for Dekalb White hens from 37 to 48 weeks reported by Silva et al. (2015). The broken line, QP max, and the intercept of the broken line and QP for egg mass from the current study is also shown Figure 4.1.

When using feed efficiency as the performance parameter, Bregendahl (2008) estimated a DLYS requirement of 693 mg/hen/d based on broken line regression. Interestingly, the broken line regression estimate for feed efficiency determined in the current study experiment was almost identical at 698 mg/hen/d.

When comparing the three regression methods, the broken line regression method estimated DLYS requirements that were lower than the QP max and intercept of the broken line and QP for all three production parameters evaluated. The highest DLYS requirements were estimated using the QP max regression. Consistently, the intercept of the broken line and QP methods yielded a requirement that was intermediate between the broken line and QP max. Similar results were observed in Chapter 3. The differences among the regression methods were expected since an earlier study showed that the broken line and QP max regressions may underestimate or overestimate the requirement, respectively, and that the intercept of the broken line and QP methods may provide a more objective intermediate value that is more economically realistic (Baker, 2002).

Comparing the three production parameters used in the current study as a group, the DLYS requirements for egg production, egg mass and feed efficiency generally agreed reasonably well with each other for the constant CP titration method. When comparing the two different titration methodologies used in this thesis (increasing CP in Chapter 3 and constant CP in this chapter), the DLYS requirement based on feed efficiency generally did not differ greatly or consistently between the two methods. However, the DLYS requirement for egg production and egg mass were consistently somewhat higher for the constant CP titration method than the increasing CP method (Table 3.12 and 4.12). The R^2 values for the egg production and egg mass regressions were higher for the increasing CP titration method than for the constant CP titration methods indicating that the former requirement estimates are more precise and may be more valid. The higher R^2 values for the increasing CP titration method likely occurred mainly because a greater response to dietary Lys level was obtained with the increasing CP method than the constant CP method.

The intercept of the broken line and QP DLYS requirement for egg mass (772 mg/hen/d) determined herein is substantially higher than the NRC (1994) requirement. The latter publication lists 690 mg/hen/d as the total LYS requirement for laying hens. With the digestibility of Lys in corn – soybean meal diets being approximately 88% (NRC, 1994), the total Lys NRC requirement value calculates to be a DLYS requirement of only 607 mg/hen/d. The higher DLYS requirement in the current study is probably due mainly to genetic differences between the hens used herein and those used in the much older studies on which the NRC (1994) requirement is based. The intercept of the broken line and QP DLYS requirement for egg mass of 772 mg/hen/d determined herein for 23 to 46 week old hens is approximately 9% higher than the DLYS value of 710 mg/hen/d recommended by the breeder for Lohmann LS Lite hens from 29 to 45 weeks (Lohmann Tierzucht, GMBH, 2014). Thus, the intercept of the broken line and QP DLYS requirement determined in the current study was somewhat, but not greatly, different than the breeder recommendation. In Chapter 3, the intercept of the broken line and QP DLYS requirement for egg mass was almost identical to the breeder recommendation. The increasing CP titration method used in Chapter 3 is more representative of practical feed formulation.

In summary, several regression methods can be used to determine a DLYS requirement for laying hens. The current study showed that for 22 to 47 week old Lohmann hens, the intercept of the broken line and QP methods yielded DLYS requirement estimates of 772, 754, and 737 mg/hen/d based on responses for egg mass, egg production and feed efficiency, respectively. When using a constant CP titration method, the intercept of the broken line and QP DLYS requirements yielded from the current study may be more economically relevant than the requirements yielded from the broken line and QP max regression. The constant CP titration method yielded more variable and less precise performance responses and DLYS requirement values than did the increasing CP titration method used in Chapter 3.

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TABLES AND FIGURE

TABLE 4.1. Ingredient and nutrient compositions of the experimental diets provided to laying hens for 0 to 12 weeks (23 to 34 weeks of age).

Ingredients	Dietary treatments 1	2	3	4	5	6	7	8
	(%)							
Corn	60.72	60.61	60.51	60.40	60.31	60.21	60.10	52.67
Soybean meal	12.78	12.78	12.78	12.78	12.78	12.78	12.78	22.22
DDGS[1]	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
MBM[1]	3.04	3.04	3.04	3.04	3.04	3.04	3.04	2.55
Soy oil	0.50	0.50	0.50	0.50	0.50	0.50	0.50	2.02
Limestone	9.45	9.47	9.48	9.50	9.51	9.52	9.54	9.47
Dicalcium phosphate	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Salt	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.28
Vitamin mix[2]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Mineral mix[3]	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04
L-Lys HCl		0.09	0.18	0.27	0.35	0.44	0.53	0.04
DL-Met	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.24
L-Arg	0.24	0.24	0.24	0.24	0.24	0.24	0.24	
L-Thr	0.29	0.29	0.29	0.29	0.29	0.29	0.29	0.03
L-Val	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.03
L-Ile	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.02
L-Trp	0.11	0.11	0.11	0.11	0.11	0.11	0.11	
Choline chloride	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Filler[4]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	
NSP Enzyme[5]	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Phytase	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Calculated composition[1]								
ME, kcal/kg	2900	2900	2900	2900	2900	2900	2900	2900
Crude protein, %	15.87	15.97	16.06	16.16	16.26	16.35	16.45	18.55
Calcium, %	4.10	4.10	4.10	4.10	4.10	4.10	4.10	4.10
Available phosphorus, %	0.44	0.44	0.44	0.44	0.44	0.44	0.44	0.44
Sodium, %	0.18	0.18	0.18	0.18	0.18	0.18	0.19	0.18
Digestible Lys, %	0.565	0.634	0.703	0.733	0.842	0.911	0.980	0.807
Digestible Thr, %	0.735	0.735	0.735	0.735	0.735	0.735	0.735	0.589
Digestible Met + Cys, %	0.900	0.900	0.900	0.900	0.900	0.900	0.900	0.726

[1]Calculated composition based on protein and amino acid analysis of the corn, soybean meal, distiller's dried grains with solubles (DDGS) and meat and bone meal (MBM).

[2]Provided the following per kg of diet: 9,370 IU of vitamin A, 3,031 IU of vitamin D_3, 28 IU of vitamin E, 0.013 mg of vitamin B_{12}. 0.088 mg of biotin, 1.9 mg of menadione, 1.9 mg of thiamine, 7.7 mg of riboflavin, 12.1 mg of dtopantothenic acid, 3.1 mg of pyridoxine, 49.6 mg of niacin, .99 mg of folic acid.

TABLE 4.1. (Cont.)

[3]Provided the following per kg of diet: 120 mg of manganese, 99 mg of zinc, 40 mg of iron, 25 mg of magnesium, 10 mg of copper, 1 mg of iodine, .3 mg of selenium.

[4]Filler=sand

[5]NSP Enzyme Econase, Cincinnati, Ohio. Phytase was provided by AB Vista, Marlborough Wiltshire, United Kingdom and was assumed to release 0.1% for available P, 0.05% calcium and 0.02% sodium.

TABLE 4.2. Ingredient and nutrient compositions of the experimental diets provided to laying hens for 13 to 24 weeks (35 to 46 weeks of age).

	Dietary treatments							
Ingredients	1	2	3	4	5	6	7	8
	(%)							
Corn	62.54	62.46	62.38	62.30	62.22	62.14	62.06	60.94
Soybean meal	8.15	8.15	8.15	8.15	8.15	8.15	8.15	14.19
DDGS[1]	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50
MBM[1]	1.93	1.93	1.93	1.93	1.93	1.93	1.93	0.39
Soy oil	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Limestone	9.62	9.62	9.62	9.62	9.62	9.62	9.62	9.75
Dicalcium phosphate	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.72
Salt	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.29
Vitamin mix[2]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Mineral mix[3]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
L-Lys HCl		0.08	0.16	0.24	0.32	0.40	0.48	0.13
DL-Met	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.20
L-Arg	0.23	0.23	0.23	0.23	0.23	0.23	0.23	
L-Thr	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.04
L-Val	0.24	0.24	0.24	0.24	0.24	0.24	0.24	
L-Ile	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.04
L-Trp	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.01
Choline chloride	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Filler[4]	2.25	2.25	2.25	2.25	2.25	2.25	2.25	
Rovabio Max[5]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Calculated composition[1]								
ME, kcal/kg	2900	2900	2900	2900	2900	2900	2900	2900
Crude protein, %	13.98	14.07	14.15	14.24	14.33	14.42	14.51	15.28
Calcium, %	4.10	4.10	4.10	4.10	4.10	4.10	4.10	4.10
Available phosphorus, %	0.44	0.44	0.44	0.44	0.44	0.44	0.44	0.44
Sodium, %	0.18	0.18	0.18	0.18	0.18	0.18	0.19	0.18
Digestible Lys, %	0.468	0.532	0.594	0.657	0.720	0.782	0.845	0.688
Digestible Thr, %	0.635	0.635	0.635	0.635	0.634	0.634	0.634	0.502
Digestible Met + Cys, %	0.779	0.779	0.778	0.778	0.778	0.777	0.777	0.619

[1]Calculated composition based on protein and amino acid analysis of the corn, soybean meal, distiller's dried grains with solubles (DDGS) and meat and bone meal (MBM).

[2]Provided the following per kg of diet: 9,370 IU of vitamin A, 3,031 IU of vitamin D_3, 28 IU of vitamin E, 0.013 mg of vitamin B_{12}. 0.088 mg of biotin, 1.9 mg of menadione, 1.9 mg of thiamine, 7.7 mg of riboflavin, 12.1 mg of dtopantothenic acid, 3.1 mg of pyridoxine, 49.6 mg of niacin, .99 mg of folic acid.

[3]Provided the following per kg of diet: 120 mg of manganese, 99 mg of zinc, 40 mg of iron, 25 mg of magnesium, 10 mg of copper, 1 mg of iodine, .3 mg of selenium.

TABLE 4.2. (Cont.)

[4]Filler=sand

[5]Rovabio Max was provided by Adisseo, Alpharetta, Georgia and it was assumed to release 0.11% for available P, 0.06% calcium and 0.03% sodium.

TABLE 4.3. Effect of increasing digestible lysine on hen-day egg production.

Diet	DLYS[1] (%)	DLYS[2] intake (mg/hen/d)	Weeks							
			2	6	10	12	14	18	22	24
			(%)							
1	0.517	452	97.5	97.4	98.0	97.6^{ab}	94.0^{b}	90.7^{c}	88.0^{b}	86.2^{b}
2	0.582	529	96.0	96.7	95.7	95.7^{b}	93.9^{b}	92.3^{bc}	88.3^{b}	88.6^{b}
3	0.648	604	95.4	96.4	96.8	96.4^{ab}	94.3^{ab}	93.9^{ab}	92.7^{a}	92.9^{a}
4	0.695	673	96.9	96.1	97.0	96.9^{ab}	94.8^{ab}	96.1^{a}	94.0^{a}	94.3^{a}
5	0.781	734	98.0	99.1	97.1	98.1^{a}	96.9^{a}	95.1^{ab}	94.8^{a}	94.9^{a}
6	0.847	802	97.6	97.9	97.0	96.7^{ab}	95.7^{ab}	95.8^{a}	94.3^{a}	93.2^{a}
7	0.913	855	97.4	97.4	97.9	97.2^{ab}	96.8^{a}	95.6^{a}	94.2^{a}	94.6^{a}
8	0.748	684	97.9	96.3	97.3	97.5^{ab}	96.7^{a}	94.2^{ab}	94.2^{a}	93.8^{a}
Pooled SEM			0.6	0.7	0.7	0.7	0.9	1.2	1.5	1.5

[1]DLYS = Digestible Lys, mean of the diets fed during Weeks 0 to 12 and 13 to 24 (Tables 4.1 and 4.2).

[2]Digestible Lys intake for Weeks 14 to 24 which was included in the regression analyses to estimate DLYS requirements.

TABLE 4.4. Effect of increasing digestible lysine on feed consumption

Diet	DLYS[1] (%)	Weeks 2	6	10	12	14	18	22	24
		(g/hen/d)							
1	0.517	95.0	96.2	101.5	99.6	100.4	97.5	93.9	93.3[b]
2	0.582	94.0	96.1	101.8	101.1	102.3	101.3	96.3	99.3[ab]
3	0.648	93.8	94.3	100.9	100.4	101.8	102.0	100.6	102.1[a]
4	0.695	94.8	94.7	101.8	100.4	102.0	104.3	101.2	102.0[a]
5	0.781	94.9	97.0	101.9	101.0	101.7	102.7	101.1	103.4[a]
6	0.847	94.3	95.5	100.9	100.2	101.2	102.7	101.9	104.1[a]
7	0.913	93.8	94.8	100.8	100.1	100.9	102.6	98.8	104.5[a]
8	0.748	94.4	95.2	101.1	99.0	98.3	100.7	98.4	100.5[ab]
Pooled SEM		0.9	0.8	1.3	1.5	1.7	2.5	3.2	2.9

[1]DLYS = Digestible Lys, mean of the diets fed during Weeks 0 to 12 and 13 to 24 (Tables 4.1 and 4.2).

TABLE 4.5. Effect of increasing digestible lysine on egg weight.

Diet	DLYS[1] (%)	DLYS[2] intake (mg/hen/d)	Weeks 2	6	10	14	18	22	24
			(g/egg)						
1	0.517	452	51.6^{c}	55.1^{b}	57.0^{b}	53.6^{c}	52.4^{d}	53.2^{d}	54.3^{d}
2	0.582	529	52.9^{bc}	55.6^{ab}	57.6^{b}	55.9^{c}	54.6^{c}	55.7^{c}	56.8^{c}
3	0.648	604	52.2^{bc}	55.4^{ab}	57.5^{b}	56.5^{bc}	55.6^{bc}	56.9^{bc}	57.5^{bc}
4	0.695	673	53.4^{ab}	56.5^{a}	57.9^{ab}	57.3^{ab}	57.5^{a}	58.0^{ab}	58.7^{abc}
5	0.781	734	53.1^{b}	55.4^{ab}	57.3^{b}	57.0^{abc}	57.5^{a}	58.2^{ab}	59.3^{ab}
6	0.847	802	53.1^{b}	56.4^{ab}	58.3^{ab}	58.0^{ab}	58.2^{a}	59.0^{a}	59.6^{a}
7	0.913	855	52.4^{bc}	55.3^{b}	57.4^{b}	57.3^{ab}	57.0^{ab}	58.0^{ab}	59.0^{ab}
8	0.748	684	54.6^{a}	55.2^{b}	59.3^{a}	58.1^{a}	58.1^{a}	59.0^{a}	58.9^{ab}
Pooled SEM			0.48	0.41	0.49	0.52	0.58	0.69	0.72

[1]DLYS = Digestible Lys, mean of the diets fed during Weeks 0 to 12 and 13 to 24 (Tables 4.1 and 4.2).

[2]Digestible Lys intake for Weeks 14 to 24 which was included in the regression analyses to estimate DLYS requirements.

TABLE 4.6. Effect of increasing digestible lysine on egg mass.

Diet	DLYS[1] (%)	DLYS[2] intake (mg/hen/d)	Weeks 2	6	10	14	18	22	24
			(g egg/hen/d)						
1	0.517	452	50.4[bc]	53.7[ab]	55.9[b]	51.4[d]	47.7[c]	47.1[c]	47.0[c]
2	0.582	529	50.8[bc]	53.8[ab]	55.4[b]	53.0[cd]	50.4[b]	49.7[bc]	50.4[b]
3	0.648	604	49.8[c]	53.4[b]	55.6[b]	53.8[bc]	52.5[b]	52.6[ab]	53.4[ab]
4	0.695	673	51.7[b]	54.3[ab]	56.2[ab]	55.0[ab]	55.1[a]	54.9[a]	55.4[a]
5	0.781	734	52.0[ab]	54.9[ab]	56.0[ab]	55.5[ab]	54.6[ab]	55.1[a]	56.3[a]
6	0.847	802	51.9[b]	55.2[a]	56.8[ab]	55.8[a]	56.0[a]	56.0[a]	55.6[a]
7	0.913	855	51.0[bc]	53.8[ab]	56.0[ab]	55.7[ab]	54.7[ab]	54.8[a]	55.8[a]
8	0.748	684	53.5[a]	55.1[ab]	57.8[a]	56.4[a]	55.2[a]	55.9[a]	55.2[a]
Pooled SEM			0.57	0.52	0.63	0.74	0.92	1.20	1.27

[1]DLYS = Digestible Lys, mean of the diets fed during Weeks 0 to 12 and 13 to 24 (Tables 4.1 and 4.2).

[2]Digestible Lys intake for Weeks 14 to 24 which was included in the regression analyses to estimate DLYS requirements.

TABLE 4.7. Effect of increasing digestible lysine on feed efficiency.

Diet	DLYS[1] (%)	DLYS[2] intake (mg/hen/d)	Weeks 2	6	10	14	18	22	24
			(g egg / g feed)						
1	0.517	452	0.530^{c}	0.558^{b}	0.557^{b}	0.514^{e}	0.491^{d}	0.503^{c}	0.505^{c}
2	0.582	529	0.540^{bc}	0.560^{b}	0.550^{b}	0.522^{de}	0.500^{cd}	0.519^{bc}	0.508^{c}
3	0.648	604	0.532^{c}	0.566^{ab}	0.557^{b}	0.533^{cd}	0.515^{bc}	0.524^{bc}	0.524^{bc}
4	0.695	673	0.546^{bc}	0.573^{ab}	0.557^{b}	0.542^{bc}	0.530^{ab}	0.543^{ab}	0.544^{ab}
5	0.781	734	0.548^{bc}	0.566^{ab}	0.554^{b}	0.549^{bc}	0.532^{ab}	0.547^{ab}	0.545^{ab}
6	0.847	802	0.550^{ab}	0.578^{a}	0.567^{ab}	0.555^{ab}	0.545^{a}	0.552^{ab}	0.534^{ab}
7	0.913	855	0.545^{bc}	0.568^{ab}	0.561^{ab}	0.554^{ab}	0.533^{ab}	0.561^{a}	0.535^{ab}
8	0.748	684	0.567^{a}	0.579^{a}	0.576^{a}	0.570^{a}	0.550^{ab}	0.571^{a}	0.550^{a}
Pooled SEM			0.0061	0.0053	0.0059	0.0055	0.087	0.0121	0.0077

[1]DLYS = Digestible Lys, mean of the diets fed during Weeks 0 to 12 and 13 to 24 (Tables 4.1 and 4.2).
[2]Digestible Lys intake for Weeks 14 to 24 which was included in the regression analyses to estimate DLYS requirements.

TABLE 4.8. Effects of increasing digestible lysine on final body weight, specific gravity, and haugh units at week 24 of experiment.

Diet	DLYS[1] (%)	DLYS intake[2] (mg/hen/d)	Final body weight (g/hen)	Specific gravity	Haugh units
1	0.517	452	1464[b]	1.0865[a]	78.71
2	0.582	529	1583[a]	1.0847[b]	77.94
3	0.648	604	1595[a]	1.0833[bc]	77.91
4	0.695	673	1606[a]	1.0839[bc]	76.74
5	0.781	734	1630[a]	1.0828[c]	77.50
6	0.847	802	1604[a]	1.0827[c]	77.43
7	0.913	855	1562[a]	1.0832[bc]	76.69
8	0.748	684	1596[a]	1.0833[bc]	76.62
Pooled SEM			26	0.0006	0.83

[1]DLYS = Digestible Lys, mean of the diets fed during Weeks 0 to 12 and 13 to 24 (Tables 4.1 and 4.2).

[2]Digestible Lys intake for Weeks 14 to 24 which was included in the regression analyses to estimate DLYS requirements.

TABLE 4.9. Effects of increasing digestible lysine on percent egg grades at Week 14.

Diet	DLYS[1] (%)	DLYS intake[2] (mg/hen/d)	Jumbo (%)	Extra large (%)	Large (%)	Medium (%)	Small (%)
1	0.517	452	0	0.89[b]	15.63[b]	71.44[a]	12.03[a]
2	0.582	529	0	1.92[b]	32.89[a]	64.22[ab]	0.96[b]
3	0.648	604	0	2.89[ab]	33.31[a]	62.90[ab]	0.89[b]
4	0.695	673	0	7.62[ab]	46.13[a]	44.32[c]	1.92[b]
5	0.781	734	0	7.55[ab]	35.30[a]	53.43[bc]	3.71[b]
6	0.847	802	0	12.91[a]	38.72[a]	46.51[bc]	1.85[b]
7	0.913	855	0.96	6.68[ab]	41.32[a]	49.10[bc]	1.92[b]
8	0.748	684	0	9.96[ab]	46.97[a]	39.32[c]	3.74[b]
Pooled SEM			0.58	3.63	5.60	5.85	1.23

[1]DLYS = Digestible Lys, mean of the diets fed during Weeks 0 to 12 and 13 to 24 (Tables 4.1 and 4.2).

[2]Digestible Lys intake for Weeks 14 to 24 which was included in the regression analyses to estimate DLYS requirements.

TABLE 4.10. Effects of increasing digestible lysine on percent egg grades at Week 24.

Diet	DLYS[1] (%)	DLYS intake[2] (mg/hen/d)	Jumbo (%)	Extra large (%)	Large (%)	Medium (%)	Small (%)
1	0.517	452	0	2.08	22.97[b]	58.44[a]	16.50[a]
2	0.582	529	0.89	6.74	37.98[ab]	50.99[ab]	3.39[b]
3	0.648	604	0	14.40	38.50[ab]	43.81[abc]	3.28[b]
4	0.695	673	1.04	19.15	44.95[a]	32.77[bc]	2.08[b]
5	0.781	734	0	19.11	49.10[a]	30.65[c]	1.13[b]
6	0.847	802	0	19.72	52.31[a]	27.96[c]	0.00[b]
7	0.913	855	0.96	16.59	50.57[a]	28.97[c]	2.89[b]
8	0.748	684	1.04	11.82	50.54[a]	35.70[bc]	0.89[b]
Pooled SEM			0.82	4.74	5.79	6.58	2.36

[1]DLYS = Digestible Lys, mean of the diets fed during Weeks 0 to 12 and 13 to 24 (Tables 4.1 and 4.2).

[2]Digestible Lys intake for Weeks 14 to 24 which was included in the regression analyses to estimate DLYS requirements.

TABLE 4.11. Effect of increasing digestible lysine on yolk percentage, yolk percentage solids, white percentage solids and total percentage solids at Week 24.

Diet	DLYS[1] (%)	DLYS Intake[2] (mg/hen/d)	Yolk %	Yolk % Solids	White % Solids	Total % Solids
1	0.517	452	33.21	50.29	11.40	24.32
2	0.582	529	32.85	49.90	11.29	23.98
3	0.648	604	33.38	50.55	11.38	24.46
4	0.695	673	33.03	50.39	11.42	24.30
5	0.781	734	33.02	50.22	11.34	24.18
6	0.847	802	33.24	49.84	11.40	24.18
7	0.913	855	33.11	50.57	11.69	24.57
8	0.748	684	32.30	50.55	11.22	24.29
Pooled SEM			0.37	0.21	0.13	0.17

[1]DLYS= Digestible Lys, mean of the diets fed during Weeks 0 to 12 and 13 to 24 (Tables 4.1 and 4.2).

[2]Digestible Lys intake for Weeks 14 to 24 which was included in the regression analyses to estimate DLYS requirements.

TABLE 4.12. Summary of the requirement methods and the digestible lysine requirements estimated using the three different regression methods.

Regression method	Egg production	Egg mass	Feed efficiency
		(mg/hen/d)	
Broken Line	686 (.55)[1]	703 (.76)	698 (.32)
QP max[2]	833 (.57)	863 (.78)	824 (.29)
Intercept of broken line and QP	754	772	737

[1]Values in parenthesis are R2 values for the regression model.
[2]QP max= the maximum of the quadratic polynomial.

FIGURE 4.1. Broken line, quadratic polynomial maximum, the intercept of the broken line and quadratic polynomial for egg mass for the constant protein diets.